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METHOD DEVELOPMENT AND MONITORING OF POLYNUCLEAR AROMATIC HYDROCARBONS IN SELECTED U.S. WATERS

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A method for concentration of trace quantities of the six representatives of polynuclear aromatic hydrocarbon (PAH) family has been developed and successfully applied to PAH monitoring in finished and raw waters.

PAH are collected by passing water through polyurethane foam plugs. Water is heated to 62 ± 2°C prior to passage and flow rate is maintained at approximately 250 ml/min to obtain quantitative recoveries. The collection is followed by elution of foam plugs with organic solvent, purification by partitioning with solvents and column chromatography on Florisil, and analysis by two dimensional thin layer chromatography-fluorometry and gas liquid chromatography-FID.

Employing this method and a sample volume of 60ℓ, PAH have been detected in all the ten water supplies sampled. Although the sum of the six representative PAH in drinking waters was small (0.9 to 15 ppt), the values found for raw waters were as high as 600 ppt. It is unclear if PAH are removed during treatment or transformed to another product and escape detection.

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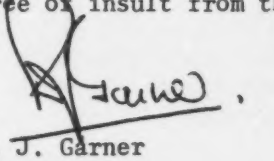
FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes the development and testing of a new and more sensitive analytical technique for monitoring polynuclear aromatic hydrocarbons in waters. With the ability to measure very low levels, we will have a better understanding of the degree of insult from these potentially harmful materials.



R. J. Garner
Director
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ABSTRACT

Flexible polyurethane foam plugs have been successfully used for concentration of trace quantities of six representatives of the polynuclear family -- fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo-(a)pyrene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene -- from drinking waters and their raw water sources. After studying a variety of parameters affecting retention, the conditions best suited for field sampling were defined. Recoveries of PAH were quantitative when water was heated to 62 $\pm$ 2°C prior to passage through foam columns. A portable sampler allowing control of optimum conditions for collection of PAH at water distribution/treatment sites was assembled. The collection of PAH on foam plugs was followed by their elution with organic solvent, purfication by partitioning with solvents, and column chromatography on Florisil. The purified concentrate was analyzed by two dimensional thin layer chromatography on cellulose acetate-alumina plates and PAH quantitated directly on the plates by fluorometry. Gas liquid chromatography - FID was also employed in the studies but failed to detect PAH in most drinking water samples and in some instances did not provide adequate resolution.

Using the method developed, analyses were performed for the 6 PAH in finished and raw waters at 10 selected water supplies in the eastern United States. PAH were detected in the ppt range in all water supplies sampled. In many cities all six representative polynuclears, as well as several unknown compounds, were detected. Although the concentration (sum of the 6 PAH) in drinking waters was small (0.9 to 15 ppt), the values found in raw water were as high as 600 ppt. whether the PAH are actually removed or transformed to some other compounds during treatment in unclear. The health significance to man of the presence of the above levels of PAH in drinking waters is not understood.

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ABBREVIATIONS AND SYMBOLS

λ	Wavelength
PAH	Polynuclear aromatic hydrocarbon
BaP	Benzo(a)pyrene
BjF	Benzo(j)fluoranthene
BkF	Benzo(k)fluoranthene
IP	Indeno(1,2,3-cd)pyrene
BPR	Benzo(ghi)perylene
PCB	Polychlorinated biphenyl
W	Watt
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
TLC	Thin layer chromatography
GLC	Gas liquid chromatography
ng	Nanogram
µg	Microgram
FID	Flame ionization detector
WHO	World Health Organization
ND	Not detected

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SECTION I

INTRODUCTION

Chemicals in the work place, in the environment, and in diet may be the single most important cause of human cancer (34). It has been estimated that at least 60% or perhaps as much as 90% of the total cancer cases in the U.S. this year will have been caused by environmental factors, mostly chemicals.

Polynuclear aromatic hydrocarbons (PAH) are of particular concern in the environment because of their demonstrated carcinogenic activity (23, 49), wide distribution and persistence in the environment (42). The potential hazards from the occurrence of PAH in man's water supplies have been noted by the World Health Organization's Committee on the Prevention of Cancer (55), which recommends evaluation of the treated surface waters for PAH. The organization has proposed that collective concentration of six PAH which serve as representatives of the whole group should not exceed 0.2 µg/ℓ.

The natural background of PAH in the environment is provided either by an endogenic synthesis of these compounds by microorganisms, phytoplankton, algae and highly developed plants or by natural pyrolytic processes, namely, forest fires and volcanic activities (50). The major source of PAH in the environment, however, arise from technological sources, such as heat and power generation, refuse burning, miscellaneous industrial processes and emission from vehicular transportation media. These carcinogenic substances may enter natural water and thereby public water supplies in a variety of ways including the release of industrial effluents, direct fall out from the atmospheric particulate matters, road run-offs, discharge of urban and domestic sewage and run-off or leaching from soils.

The studies on the incidence of PAH in the water environment have been predominantly carried out in Europe and are pertinent to European waters only. A number of PAH have been detected in European natural and treated waters at levels which are alarming. In one instance, the concentration of benzo(a)pyrene alone was 0.01 mg/ℓ in drinking water and as high as 6 mg/ℓ in strongly polluted surface water (52). Data regarding their levels in U.S. water is virtually nonexistant. In view of the fact that polynuclear compounds have been detected in the air of U.S. urban and non-urban sites (36) and in soil and marine sediments (4, 5), it is suspected that they may also be present in U.S. waters. The evaluation of hazards to the public from the presence of PAH in water and the implementation of the remedial measures requires the knowledge of the levels and the nature of PAH in these waters.

The objectives of the present study were to determine the concentration of PAH in a selected raw water source used for drinking purposes and their degree of removal as a result of the treatment processes. Since no suitable method was available for concentration of PAH from water, the first phase of the project was devoted to developing a rapid and efficient method for the preconcentration of trace quantities of PAH. The second phase consisted of developing an analytical scheme for the quantitation of collected PAH, and monitoring of PAH in drinking waters and their raw water sources.

SECTION II

GENERAL CONCLUSIONS

Polyurethane foams under optimum conditions can be used to concentrate trace quantities of polynuclear aromatic hydrocarbons from water. The inability of the earlier investigators to recognize and use the sorbent properties of polyurethane foam was due to the absence of a systematic study dealing with various parameters affecting retention. The method developed enables sampling of larger volumes of water, and is more efficient and practical than cumbersome batch extraction methods normally used. The method of concentration can be conveniently used at the sampling site and thus eliminates the need for sample preservation and transport, and problems arising from adsorption on the container. These advantages render this method very desirable for PAH analysis in water.

The chemical clean up of the foam eluate utilizing solvent partitioning and column chromatographic separation was found to be necessary prior to subjecting to analysis. Final purification and resolution of the sample concentrate on two dimensional thin layer chromatography was found preferable over GLC columns. For identification and quantitation, fluorometric analysis was more suitable than flame ionization detection. The former method was more sensitive and selective and possessed a much larger sample capacity, allowing a lower detection limit. With overall preconcentration (60ℓ volume), clean up and detection procedure, concentrations of PAH as low as 0.1 ppt can be determined.

Judging from the limited information generated, PAH appear to be widespread in drinking waters and their raw water sources, although concentrations, particularly in drinking waters, are low. Current technology of water treatment is able to substantially reduce the levels of PAH in treated waters. Whether the reduction is due to removal or transformation to other products could not be concluded from this study.

SECTION III

RECOMMENDATIONS

It is recommended that further research in the following areas be undertaken to fully understand the implications of the presence of polynuclear aromatic hydrocarbons in drinking waters and their raw intake waters:

A. Our results of single samples from a few water supplies reveal that PAH are widespread in raw and drinking waters although their levels are generally low. More monitoring work using the sensitive method developed must be undertaken to derive more definite conclusions.

B. Work in Germany has shown that PAH are introduced in water during distribution through paint, asphalt and other such material used for coating the pipes. Thus, studies should be undertaken to evaluate the additional contribution of PAH, if any, arising from the supply network.

C. Our determinations of the levels of PAH in finished and raw waters have shown that considerable reduction in the concentration of PAH takes place in water treatment. Whether this decrease reflects a true removal or transformation of PAH to other and perhaps more hazardous products must be ascertained.

D. The cumulative cancer risk to humans posed from the presence of low levels of PAH in drinking waters can not be assessed by conventional animal tests. Thus, epidemiological studies must be undertaken. Alternatively, studies employing more sensitive *in vitro* test methods must be carried out. One must keep in mind that these compounds may possess a very high bioaccumulation potential.

E. It is well known that the particulate PAH emission is maximum during winter months because of home heating. This, coupled with the fact that microbial breakdown of PAH, if any, will be lower during winter months, may result in increase in PAH concentration during winter months. On the other hand, concentration during summer months may rise due to increased solubility of PAH. Thus, the fluctuations in the levels of PAH due to seasonal variations must be studied.

F. To establish a "cause and effect" relationship, simultaneous monitoring of effluents from discharge sites and the drinking waters derived from raw waters receiving such discharges should be performed.

G. As regards the monitoring of PAH in drinking waters, although routine monitoring at every location may not be necessary, it is recommended that at least one center be set up to carry out the monitoring of PAH using the present method. Training of the treatment plant personnel to use the concentration procedure will help reduce the overall cost of such a program.

It is also recommended that an average ratio of benzo(a)pyrene to other carcinogenic PAH be established from the analysis of a large number of drinking waters. Subsequently, it may be possible to monitor for benzo(a)pyrene alone, and derive the overall concentration of carcinogenic PAH by multiplying with the factor.

H. The ability of polyurethane foam plugs to collect PAH quantitatively upon heating of the water, also opens up a variety of new areas for further research. Retention of contaminants such as PCB's, pesticides, phthalate esters and others will probably also be enhanced from water at elevated temperatures, and should be examined. The high sorbent ability and low cost suggests the feasibility of polyurethane foam filtration as an effective method of water treatment. Polyurethane foam may also serve as an alternate to the carbon adsorption method for monitoring trace organic contaminants in water. Efficient collection of non-particulate polycyclic organic matter from air may also be possible with polyurethane foam. Further evaluation of polyurethane foam is necessary for these purposes.

SECTION IV

LITERATURE REVIEW

The first phase of the project was devoted to updating the literature relating to the occurrence of PAH in environmental waters, their degree of removal and/or destruction by various treatment processes, and the method of collection and analysis of PAH. The review of collection methods included various techniques of concentration of trace organics from water with particular emphasis on polyurethane foam method. Analytical methods for separation and identification of PAH from different sources, including atmospheric particulate matters, water environment, soils and sediments, were extensively reviewed. Some areas were searched by computerized literature search services, whereas in other cases manual review was undertaken. The result of the review work is reflected by the different pertinent references cited in various sections of this report.

SECTION V

PRECONCENTRATION METHOD

BASIS FOR DEVELOPING PRECONCENTRATION METHOD

The difficulty in identifying and quantitating trace quantities of PAH often encountered in drinking water necessitates a preconcentration step to meet detection limit of the analytical method. The methods available at the present time are unattractive because of poor recovery, and their inability to handle large sample volumes. Therefore, the initial study of the monitoring phase was devoted to the development of a suitable preconcentration method.

Liquid-liquid extraction is the most widely used method for the preconcentration of organic compounds from water. Although the method is employed for the analysis of PAH in water samples, it is rendered unfeasible when the volume of the sample becomes large. Instruments employing on-site continuous liquid-liquid extraction (2) have doubtful practical value for routine analysis of PAH because of slow flow rates (20-30 ml/min) necessary to establish an equilibrium distribution between the aqueous and organic phases. The disadvantage of both direct and continuous solvent extraction methods is that many good PAH solvents (e.g. benzene) cannot be used in the system because of their relatively high water solubility.

Concentration of PAH on a suitable sorbent offers a viable alternative. The two most promising sorbents presently available, e.g., XAD-resin (11, 53) and Tenax (31) have limited value because of their incapability of allowing water flow rates greater than 50 ml/min. Passage of large volumes of water at such flow rates is very time consuming.

The discovery of the ability of polyurethane foams to retain a number of compounds including PCB's and organochlorine pesticides from water (3, 8, 15) produced a surge of interest in using this technique to concentrate other compounds as well. Gough and Gesser (17) used polyurethane foams for the recovery of phthalate esters from water. These authors and others (51, 35) used foam plugs coated with gas chromatographic liquid phases with varying degrees of success. Bedford (3), however, reported that polyurethane foams cannot be used reliably to extract PCB's from turbid natural waters. The studies conducted by EPA (53) with paper mill wastewater components, fuel oil, and textile dyes showed that both coated and uncoated foams are very limited in their extraction ability.

Detection of very low concentrations of PAH in drinking water requires that the preconcentration method devised should be able to deal with a large volume of samples in a relatively short time. Preconcentration using flexible polyurethane foams meets the above characteristics and is the method described in this report. The optimum conditions for PAH retention by foam plugs were first determined with radioactive benzo(a)pyrene for ease of detection. The method was then extended successfully to five other PAH to demonstrate its general applicability as a method of preconcentration of PAH.

EVALUATION OF FOAM PLUGS FOR EXTRACTION AND RECOVERY OF BENZO(A)PYRENE (BaP)

The initial evaluation of foam plugs for the suitability of extraction of various PAH from large volume of water was conducted with BaP, one of the representative PAH. Radiolabelled BaP (7-10-^{14}C) was used in these studies because of ease and greater sensitivity of ^{14}C-detection. This also eliminated the necessity for determination of background levels of BaP in water. It was assumed that if the preconcentration method proved successful with BaP, the method could be extended to other PAH as well.

Materials and Reagents

Radiolabelled BaP (7-10-^{14}C): Radiolabelled BaP was obtained from California Bionuclear Corporation with a manufacturer's claimed purity of 98%. Storage of this solution showed an impurity spot on benzene developed cellulose TLC plates as evidenced by scanning for radioactive spots on a Nuclear Chicago Actigraph III. Purification by partitioning between benzene-water (3:7) phase removed this impurity.

Polyurethane Foam Plugs: Flexible polyurethane foam plugs, and sheets from which appropriate size plugs were cut, were obtained from commercial sources. The types of foams used in the study and their sources are summarized in Table 1.

TABLE 1. CHARACTERISTICS OF VARIOUS TYPES OF FOAMS USED

Source	Trade Name	Chemical Nature	Color	Plug Dimension (diameter x length, mm)	Foam Density (kg/m^3)	Referred to in text as
Scientific Products, Inc. (Batch purchased in 1974)	DiSPo plugs	Polyester	White	50 x 38	~24	A
Scientific Products, Inc. (Batch purchased in 1976)	DiSPo plugs	Polyester	White	50 x 38	~22	B
VWR Scientific	Identi plugs	Polyester	White	45 x 45	~25	C
Thomas E. Forrest Co., Inc	UU34	Polyester	Green	45 x 45*	24	D

* Plugs were cut from 45-mm thick flexible polyurethane foam sheet.

Sources of Water: Tap water used in these studies was obtained from laboratory tap and is derived from Skaneateles Lake. The only treatments the water received prior to distribution were chlorination and fluoridation (37). Raw water was collected from Onondaga Lake (Syracuse, N.Y.) in the month of December. Large floating and suspended particles were removed by filtration through a fine wire screen prior to use.

Chromaflex Columns: Extender type columns of i.d. 20, 25, 40 and 50 mm with "O" rings, adapter and clamps were purchased from Kontes Glass Co.

Oscillating-type pump, Gilmont flowmeter, and Haake Model FE thermostated circulator with 1000 W. heater: These were purchased from Scientific Products, Inc.

Radioactive Counter: The radioactive counting was done with the aid of a Nuclear-Chicago 720 series automatic liquid scintillation system.

Radioactive Scanner: Scanning of radioactive spots on a strip of TLC plate was performed by a Nuclear Chicago Actigraph III.

Chemicals: All solvents used were A.R. grade and purchased from Mallinckrodt Chemical Co. The nematic liquid crystal [N,N'bis(p-methoxybenzylidene)-α,α'-bi-p-toludine] was obtained from Eastman Kodak Co. and gas chromatographic phases DC-200 and SE-30 from Analabs, Inc. Insta-gel and the chemicals for preparing scintillation fluid were purchased from Packard Instrument Co.

Procedure

The ability of various foam plugs to retain BaP from aqueous solution under static conditions was determined by allowing preweighed foam plugs to equilibrate for 4 hours in 150 ml volumes of ^{14}C-BaP solution of different concentrations. The activity of the initial and final solution was determined by counting a 5 ml aliquot of the water in Insta-gel. From the difference in the ^{14}C-activity in initial and final water sample, the amount of ^{14}C-BaP sorbed by foam plugs was calculated. The adsorption of BaP on the container was taken into consideration by allowing equilibration of the solution with the glass container prior to introducing the foam plugs and taking the residual activity in water as the initial BaP concentration.

In flow system experiments, a foam plug was wetted with distilled water, squeezed to expel air and placed in a Chromaflex column. Each plug was washed with 20 ml acetone, 50 ml benzene, again 20 ml acetone, and finally with 250 ml distilled water. In a typical experiment, four liters of water was spiked with ^{14}C-BaP to a concentration of 0.1 ppb and allowed to stand for 30 minutes to allow equilibration with glass surface. The equilibrated solution was drawn through the column at a constant flow rate of 250 $\pm$ 10 ml/min by means of two oscillating type pumps connected in series and controlled by a Variac. The flow rate was continuously monitored with the help of a Gilmont flowmeter. Prior to reaching the foam column, the water was brought to a desired temperature by passing it through a custom made glass coil (25 cm x 6 mm) which was immersed in a Haake thermostated circulator.

The coil was placed inside the reservoir housing through the opening of the cover plate. For continuously monitoring the temperature of water during passage through the column, a water trap equipped with a thermometer was introduced in the line between the exit end of the column and the pump. The complete set-up is shown in Figure 1.

The concentration of radioactive BaP in spiked water and effluent after passage through the foam plug was determined by extracting a known aliquot (100 and 500 ml, respectively) with 20 ml benzene and counting the ^{14}C-activity in the solvent layer. To account for change in the concentration of BaP in spiked water with time, two samples, one at the beginning and the other at the end of the run, were taken and the values were averaged.

The BaP retained on the foam was eluted with 20 ml acetone followed by 75 ml benzene. In experiments where two foams were used in the same column, the volume of eluent acetone and benzene was increased to 30 and 125 ml, respectively. Soxhlet extraction of the plugs did not elute more BaP than that recovered by batch column extraction and, therefore, its use was not considered necessary. The calculation of the efficiency of retention of BaP on the foam plugs was based on the concentration of BaP detected in spiked water.

The amount of BaP retained on the glass bottle, heating coil, and connecting tubings was determined by washing them individually with enough acetone necessary to avoid emulsion formation and enough benzene to leach out all the activity.

In every case a 5 ml aliquot of the extracted solution was used for radioactive counting with 10 ml of scintillation fluid. The scintillation fluid was composed of 5 g of 2,5-diphenyloxazole (PPO) and 0.15 g of 2,2'-phenylenebis-(5-phenyl)oxazole (POPOP) per liter of toluene. Water samples were counted, when necessary, in Insta-gel.

In several experiments the foam plugs were coated with selective chromatographic phases according to the procedure of Uthe *et al*. (51), and tested for their sorption ability towards BaP.

The stability of BaP on foam plugs was studied by passing 2 liters of BaP spiked tap water (0.1 ppb) through the foam plugs and storing the plugs with sorbed BaP for various lengths of time. In order to prevent photodegradation of BaP, the plugs were stored in Chromaflex columns covered with aluminum foil. Following the storage periods, BaP from the foam plugs was eluted as before and counted for ^{14}C-activity. The eluent from the storage experiments was concentrated and subjected to thin layer chromatography for determining if any degradation of BaP had occurred. Cellulose plates were spotted with the concentrate and developed with benzene as solvent. The plates were scanned for radioactive spots on a Nuclear Chicago Actigraph III.

Most experiments were repeated 2-3 times and results are expressed as mean of these values. The deviations from the mean were usually in the range of 3-6%.

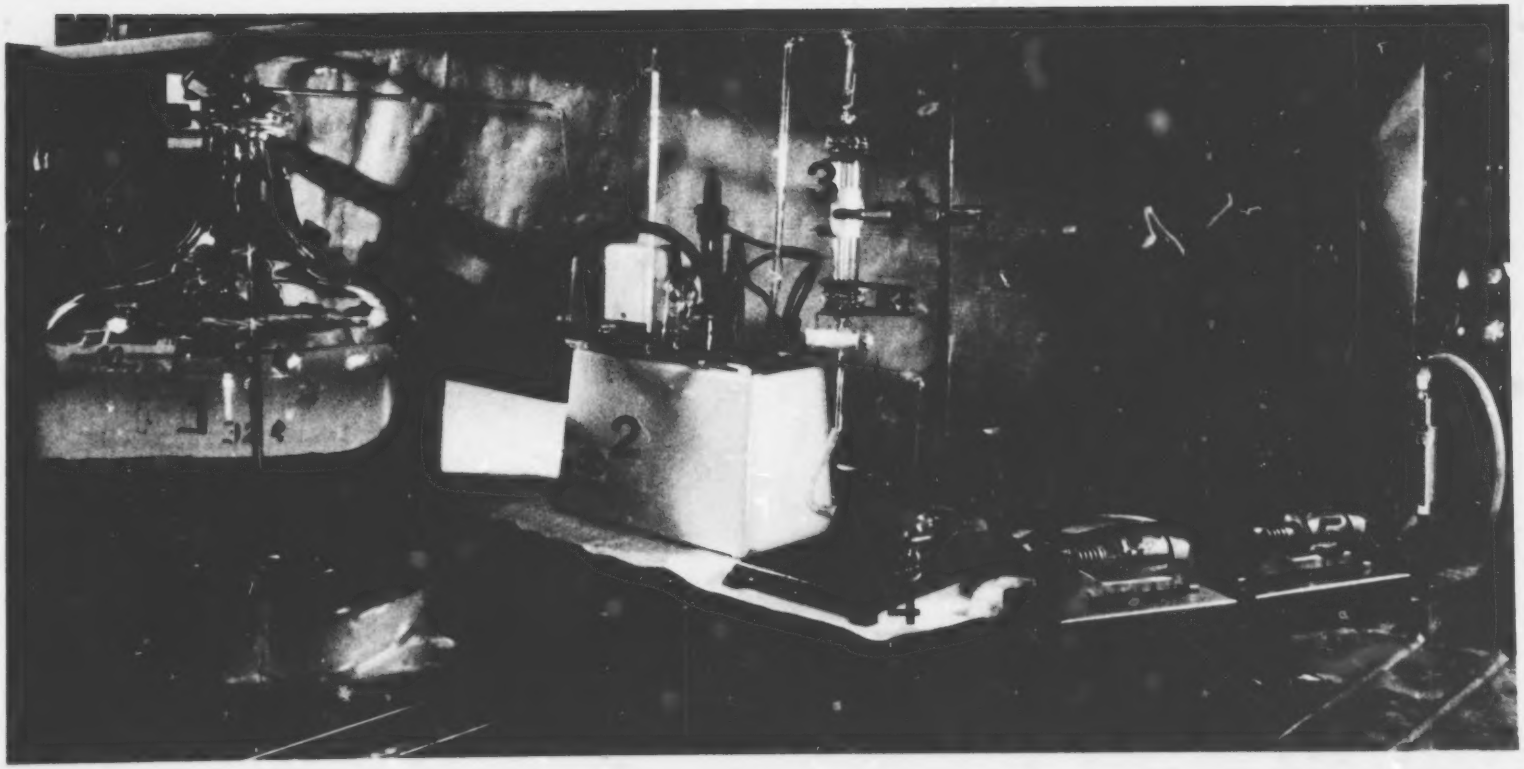


Figure 1. Set-up for flow system extraction: 1 reservoir, 2 thermostated water bath, 3 foam column, 4 water trap for measuring temperature, 5 pump, 6 variac, 7 flowmeter.

Results and Discussion

Sorption Characteristics Under Static and Flow Condition--

Preliminary assessment of retention characteristics of various foam plugs was based on the ability of the plugs to retain BaP from aqueous solution under static conditions. Musty and Nickless (35) used methylene blue adsorption from aqueous solution for determining the relative effectiveness of foam plugs for removing pesticides. A more direct method, such as used in the present case, has been used by Lawrence and Tosine (29).

The retention of BaP by various foam plugs increased linearly with increase in BaP concentration as shown in Figure 2. The foam plugs differed only to a small extent in their sorption characteristics under static conditions. It was found that type D foam sorbed the greatest amount of BaP, followed by foams B, C, and A. The polymer linkage in the foam-ester or ether, and foam density did not appear to be related to the sorption properties.

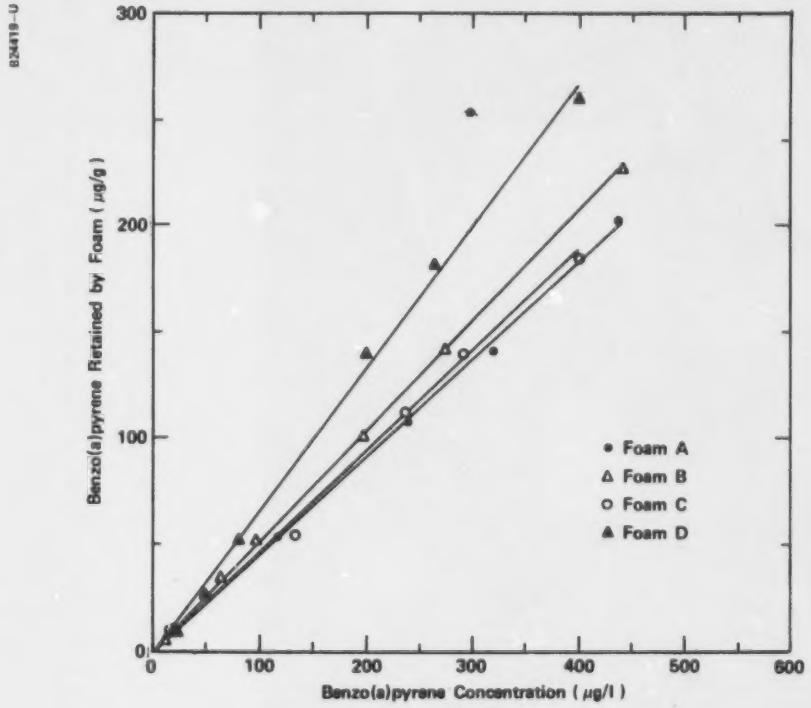


Figure 2. Sorption of BaP by various foam plugs under static conditions.

The results presented in Table 2 show the recoveries of BaP from 4 liters of spiked-distilled and tap water obtained with various foam plugs in a continuous flow system. The results are in agreement with the recoveries obtained under static conditions. Foam type D was most effective in retaining BaP and again, the retention abilities of various foam plugs were within a narrow range. The recovery from tap water with various foam plugs at ambient temperature ranged between 58-66%. Under similar conditions the recovery from spiked-distilled water was considerably higher (89-96%). The decreased retention from tap water was probably not due to competition for sites by contaminants since the retention of BaP from distilled water was not affected by prior exposure to tap water. The lower efficiency appeared to be linked to the presence of suspended particles since the tap water dosed with BaP following Millipore filtration (0.45μ) gave retention values equivalent to distilled water.

TABLE 2. RECOVERY OF BaP FROM SPIKED WATER BY CONTINUOUS FLOW SYSTEM: WATER TEMPERATURE, 23°C; VOLUME, 4ℓ; FLOW RATE, 150 ± 10 ml/min; COLUMN DIAMETER, 25 mm.; BaP CONCN., 0.1 ppb

Foam plug	% Recovery from	
	Tap water	Distilled water
A	62	89
B	58	91
C	65	91
D	66	96

Other Considerations in Selection of Foam Plugs--

The water flow rates from foam plugs of type B were found not to be uniform from plug to plug. With tap water the maximum flow rates did not exceed 150 ml/min. An effort was made to increase flow rates of these plugs. The foam plugs were subjected to treatment in alkaline solutions according to the procedure of Buist and Gudgeon (10) to clean foam cells by removing face membranes. Such treatment of type B plugs, although increasing water flow rates, resulted in reduction in BaP sorption capability. The possibility of using shredded foam in place of foam plugs was also investigated. The foam plugs were shredded with the help of a Virtis tissue homogenizer, packed loosely in a 25 mm column, and used in the retention studies. The shredded foam column allowed high flow rates and gave a good BaP retention efficiency. The efficiency, however, depended heavily on the technique of the packing of the column, and a slight variation resulted in marked decrease in BaP retention efficiency. In view of this difficulty, the use of shredded foam was not considered further.

The type D plugs were colored green and the possibility that the colored material may be eluted and interfere with the determination of PAH led to their exclusion from further use. Plug type A were no longer commercially available. Based on the above considerations, type C plugs were selected for further studies. These plugs showed good water flow rates and sorption characteristics for BaP from spiked tap water.

Parameters Affecting Sorption Characteristics--

The following parameters have been studied with respect to their affect on sorption characteristics.

BaP retention as a function of flow rates--The efficiency of foam plugs to retain BaP at water flow rates in the range of 130 to 520 ml/min was examined. As shown in Figure 3, the recovery remained unaltered with change in flow rates with both distilled and tap water. This is particularly encouraging since the adjustment of the flow rates will not be critical under most experimental conditions, as long as the rates stay within broad limits.

Even though BaP recovery was independent of flow rates in the range examined, a flow rate of 250 $\pm$ 10 ml/min was preferred for further studies. At flow rates exceeding this value, the foam plug quite often began to slide down and rest at the bottom of the glass column during the runs. Foam plugs pressed against the bottom of glass columns in this manner retained BaP less effectively.

A further study was carried out to determine the desorption of BaP from foam with tap water. Various volumes of tap water were passed over spiked foam plugs, and the amount of BaP remaining on the foam was determined. The findings revealed no significant removal of already sorbed BaP from foam regardless of the volume of water passed and the flow rates.

Effect of column diameter on BaP retention from tap water--The results of increasing the diameter of the column holding the plugs on BaP recovery from tap water is shown in Table 3. As the column diameter is increased, (20-50 mm), the recovery of BaP on the foam plug steadily decreased; the value with water at ambient temperature changing from 53% for a 50 mm column to 73% for a 20 mm column. Squeezing of the foam into a small column probably results in the reduction of foam pore size, and subsequently in more effective retention of the particulate-sorped BaP.

Although the column with 20 mm diameter allowed the highest recovery of BaP from tap water, the squeezing of the foam in a smaller diameter column produced difficulty in attaining flow rate of 250 ml/min. An approximate ratio of 2:1 between the plug and column diameter is considered the best compromise between retention efficiency and flow rate. Accordingly, the foam plugs of 45 mm diameter were held in a 25 mm column in the studies described in this report.

Effect of pH on BaP retention from tap water--The effect of pH of the spiked water on the recovery of BaP on the foam plugs is shown in Figure 4. As can be seen, a significant increase in the retention efficiency of foam occurs with an increase in pH. At ambient temperature the retention at tap

Table 3. EFFECT OF DIAMETER OF COLUMN USED FOR HOLDING THE FOAM PLUG ON BaP RETENTION FROM TAP WATER: WATER TEMPERATURE 23°C; VOLUME, 4ℓ; FLOW RATE, 250 ± 10 ml/min; BaP CONCN., 0.1 ppb

Column diameter (mm)	% Recovery of BaP
20	73
25	65
40	62
50	53

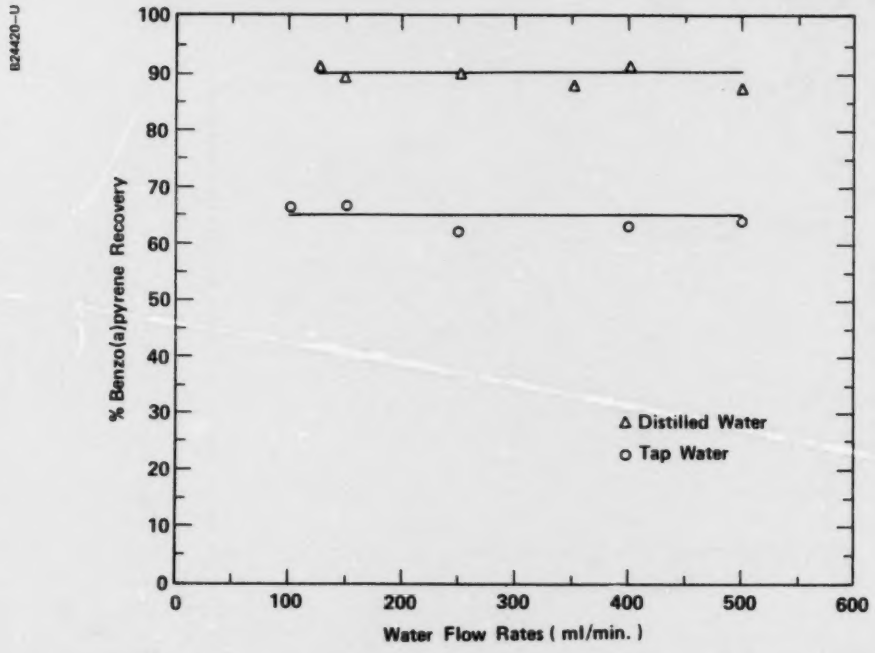


Figure 3. Retention of BaP from spiked tap water at various flow rates: water temperature, 23°C; volume, 4ℓ; BaP concentration, 0.1 ppb.

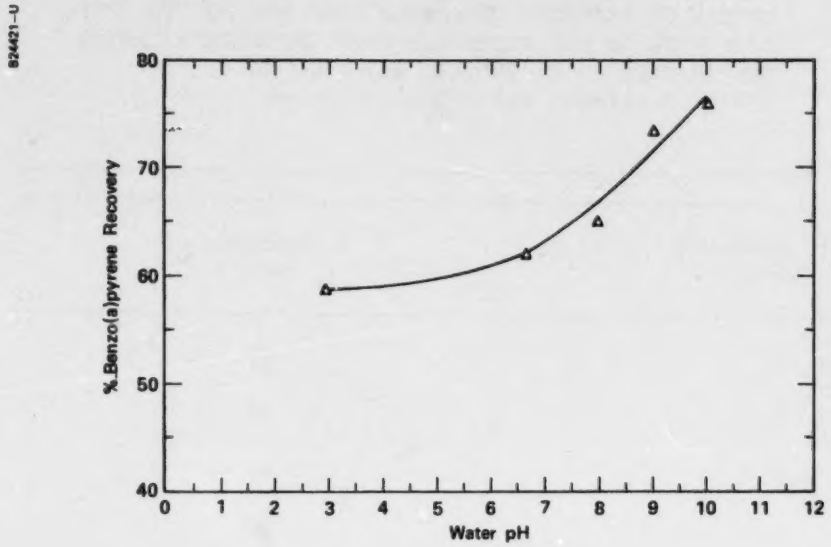


Figure 4. Influence of water pH on BaP retention: water temperature, 23°C; volume, 4ℓ; flow rate, 250 ± 10 ml/min; BaP concentration, 0.1 ppb.

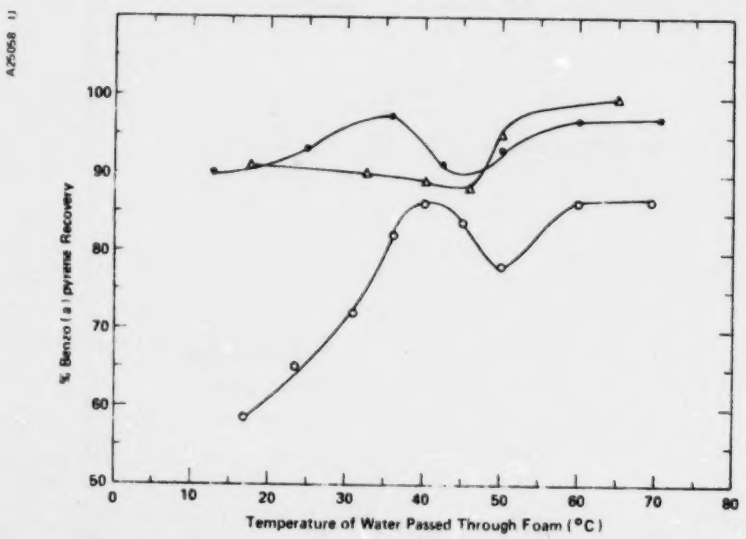


Figure 5. Effect of water temperature on recovery of BaP on foam plugs: volume of spiked water, 4ℓ; BaP concentration, 0.1 ppb; flow rates, 250 ± 10 ml/min. o = tap water (unfiltered), • = filtered tap water, Δ = distilled water.

water pH (6.7) was between 62-65%, but upon increasing the pH to 10.0, the retention efficiency increased to 76%. Lowering the water pH below 6.7 resulted in a decrease in BaP retention. A pH-linked increase in the recovery of chlorinated hydrocarbons on polyurethane foam plugs has been noted by Musty and Nickless (35).

The increase in retention with increase in pH may be attributed to the desorption of BaP from the suspended particles and subsequent sorption on the foam plug, or to coagulation of suspended particles and minerals in water at higher pH enhancing retention through filtration. Alternatively, a pH-dependent increase in the sorption process in the foam may be responsible for increased retention of BaP. If a charge-transfer complex formation is involved between the foam polymer and BaP, such an increase is not improbable.

Effect of water temperature on BaP recovery--The most dramatic effect on the recovery of benzo(a)pyrene from spiked tap water was observed when the temperature of the water was varied (Figure 5). The relationship between BaP recovery from tap water and temperature was found to be biphasic. The percent retention steadily increased with increase in temperature up to 40°C, but decreased with further increase in temperature. When the temperature was increased beyond 50°C, the increase in BaP retention was resumed until a plateau was reached starting at 60°C. The recovery of BaP at temperature > 60°C was approximately 87%.

The effect of temperature on BaP recovery from tap water is complex and probably the consequence of many interacting factors. The initial increase appears to be linked to the presence of suspended particles in water. This is due to the fact that such an increase was not seen with spiked distilled water, and that the increase was less pronounced when tap water was Millipore-filtered prior to spiking. It is suggested that heating of tap water causes desorption of BaP from suspended particles and thus prevents loss of BaP which otherwise occurs due to passage of particle-sorbed BaP through the porous foam.

The increased retention of BaP beyond 50°C is observed with tap water, distilled water, as well as filtered water and, therefore, appears to be linked to the foam itself. Such an increase could be attributed to a possible change in the conformation of the polymer at higher temperature, thereby increasing sorption of BaP.

Subjecting of polyurethane foam to the action of hot water or steam causes hydrolysis of residual isocyanate and ethyl silicate (47). The increased retention of BaP at higher temperature may be linked to these chemical changes in the foam. However, this did not appear to be the case, since the pre-heated or steam-treated plugs gave recovery of BaP similar to untreated foam. The data suggests that the increase in BaP sorption at higher temperature is not due to chemical change in the foam, but probably to a reversible conformational change.

Effect of coating foams on BaP recovery--The retention ability of foam plugs for many pesticides has been shown to be improved by coating the plugs

with selective sorbents (51). Studies were undertaken to determine if coating of the plugs with suitable chromatographic phase can also improve the retention of BaP from water. The chromatographic phases tested included nematic liquid crystal, SE-30 and DC-200. Both nematic crystal and SE-30 have been used earlier as gas chromatographic phases for separation of PAH compounds (24, 45). DC-200 was also tested because Uthe et al. (51) reported good recoveries of pesticides with foam plugs coated with this phase. With the foams coated to the extent of 5-10% by weight, an increase in BaP recovery of 4-9% over untreated foam was observed at a water temperature of 62°C ± 2 (Table 4). The eluate from coated-foam plugs contained large quantity of the coating material and its concentration to smaller volume (which would be necessary in the later stages of development) was difficult. Furthermore, since only a small increase in BaP retention was noted in the presence of the chromatographic phases, the coating of the foam plugs was not considered further.

TABLE 4. BENZO(a)PYRENE RETENTION FROM TAP WATER WITH FOAM PLUGS COATED WITH CHROMATOGRAPHIC PHASES: WATER VOLUME 4ℓ; FLOW RATE, 250 ± 10 ml/min; BaP CONCENTRATION, 0.1 ppb

Chromatographic phase	Concentration of coating on plug (% of foam, w/w)	Temperature of spiked water (°C)	% Retention on foam
Uncoated	-	23	62.0
Uncoated	-	62	85.3
DC-200	5	62	87.5
DC-200	10	62	92.6
SE-30	10	62	91.2
Nematic liquid crystal [N,N'-bis(p-methoxybenzylidine)-α,α'-bi-p-toludine]	3.7	23	66.0

Effect of BaP concentration on recovery--In order to become an effective method of preconcentration, the foam plugs should demonstrate high and consistent recovery at varying concentrations of BaP usually encountered in treated and raw waters. In view of this, the recovery studies with the foam plugs were carried out at different concentrations. The results of the study are shown in Table 5. The retention of BaP did not vary significantly with change in BaP concentration in the range examined (0.002-25 ppb). This can be interpreted to mean that the polyurethane foam plugs of the dimension and chemical characteristics used in this work can effectively concentrate BaP from tap water over a broad concentration range.

TABLE 5. RECOVERIES OF BaP FROM SPIKED TAP WATER AT VARIOUS CONCENTRATIONS: WATER VOLUME, 4ℓ; FLOW RATE, 250 ± 10 ml/min; TEMPERATURE, 62 ± 2°C

Concn. of BaP* (ppb)	% Retention of foam
25	84.0
0.1	86.0
0.05	84.0
0.02	83.0
0.002	87.0

*On the basis of the amount added to water

Mass Balance of ^{14}C-Activity--

Benzo(a)pyrene, like other PAH, has a tendency to stay adsorbed on solid surfaces. Considerable amount of BaP added to water can be expected to be adsorbed to the wall of the reservoir, glass coil and connecting tube, etc., employed in the experimental set-up. Experiments were carried out to determine complete mass balance of the BaP added to water to account for losses due to sorption on various surfaces. The studies were carried out at 62 ± 2°C with tap as well as distilled water.

As can be seen in Table 6, nearly 25% of the total ^{14}C-added to tap water was recovered from the bottle surface. From distilled water, however, the loss to the bottle was only 15%. The extractability of BaP with benzene from the two types of water also varied to a significant extent. All the BaP was extractable with benzene from spiked distilled water. The amount recoverable from tap water was only 92%. Whether this is due to transformation of BaP to some other form not extractable with benzene or due to non-extractable particle-adsorbed portion of BaP is not clear. Acheson et al. (1) have suggested that adsorption of PAH upon suspended solids may lead to a change in extraction efficiency.

The calculation of the percent retention of BaP by foam plugs in this report has been based on the amount of ^{14}C-detected in spiked water by benzene extraction. This eliminates the necessity for quantitation of the BaP adsorbed on the reservoir surface. The total loss of BaP due to adsorption on the glass coil used for heating of the water, and on connecting tubes was small, and ignored in the calculation of the percent retention efficiency.

TABLE 6. MASS BALANCE OF ^{14}C-ACTIVITY ADDED TO WATER: WATER VOLUME, 4ℓ, TAKEN IN A 5ℓ BOTTLE; FLOW RATE, 250 ± 10 ml/min; TEMPERATURE, 62 ± 2°C; BaP CONCENTRATION, 0.1 ppb

Material Tested	Tap Water		Distilled Water	
	%^{14}C-distribution		%^{14}C-distribution	
	of the amount added to water	of the amount detected in water*	of the amount added to water	of the amount detected in water*
Foam plug + glass column	56.0	86.0	80.0	95.0
Glass Bottle	24.4	-	15.0	-
Glass coil and connecting tubes	2.1	2.8	1.8	2.1
Effluent	8.0	11.0	3.2	3.3
^{14}C-Non-extractable	8.0	-	0	-
Total	98.5	99.8	100	100.4

*by extraction with benzene

Determination of BaP Breakthrough Volume With Tap Water--

Efforts were made to determine the volume of water from which a single foam plug could concentrate BaP efficiently. Increasing volumes of spiked water were passed through individual foam plugs and the efficiency of BaP retention was determined in each case. The results showed that the efficiency of retention steadily declined as the sample volume increased; the efficiency of retention with 4ℓ of tap water was nearly 86%, however, when the sample volume was increased to 40ℓ the efficiency fell to nearly 50% (Table 7). The efficiency of retention from distilled water, however, remained > 95% even at sample volume of 40ℓ.

TABLE 7. EFFECT OF SAMPLE VOLUME ON THE RECOVERY OF BaP WITH A SINGLE FOAM PLUG. FLOW RATE, 250 ± 10 ml/min; TEMPERATURE, 62 ± 2°C; BaP CONCN., 0.05 ppb

Description of water	Sample volume (ℓ)	% BaP retention
Tap water	4	86.5
	5	84
	10	73
	20	67
	40	49
Distilled water	4	98
	40	95

In an attempt to increase the efficiency of retention with larger volumes of water, the number of foam plugs in the column was increased. With 20ℓ of spiked-tap water, 4 plugs - two each in two different columns - gave a recovery of 85.5%. The distribution of BaP retained on individual plugs was as follows: 65% on the first plug, 4% on the second, 13% on the third (first plug on the second column), and 3.5% on the fourth.

BaP Recovery from Spiked Surface Waters--

It was of interest to determine if polyurethane foam plugs can effectively concentrate BaP from raw water as well. Onondaga Lake water was chosen as a case of raw water as it would represent a worst possible case of raw drinking water source (total suspended solids in water = 102 mg/l; total solids = 2.4 g/l). The retention from 4ℓ of spiked raw water (0.1 ppb) with a single foam plug was found to be 69%. This indicates that breakthrough occurs earlier for raw water than tap water. When the number of plugs in the column was increased to two, the efficiency of retention resumed to normal value. Therefore, twice as many total foam plugs (not exceeding two plugs per column) should be sufficient to effectively concentrate BaP from the same volume of

raw water as with tap water. Since the concentration of BaP in raw water can be expected to be higher, the number of foam plugs required can be cut down by decreasing the sample size.

Stability of BaP on Foam Plugs--

Prior to considering foam plugs for field monitoring, it is important to assess the stability of BaP on foam plugs. The effect of storage at room temperature and in refrigerator was compared over a 7 day period. Almost all the ^{14}C-activity was recoverable from foam plugs after 7 days storage at room temperature (Table 8). Thin-layer chromatography of the eluates from foam plugs stored at room temperature and at 4°C, revealed no measurable ^{14}C-activity in any spot other than BaP. The data suggests that BaP is sufficiently stable on foam plugs for transportation to the laboratory for analysis. However, cooling of the foam plugs to 4°C during transportation is suggested.

TABLE 8. STABILITY OF BaP ON FOAM PLUGS STORED AT ROOM TEMPERATURE AND AT 4°C. EACH PLUG WAS SPIKED WITH APPROXIMATELY 0.2 µg BaP

Storage temperature	% recovery of BaP at different storage periods (days)			
	1	2	4	7
4°C	100	91	103	95
Ambient	98	90	87	82

Conclusion

Polyurethane foam plugs have been found to be excellent sorbent for benzo(a)pyrene from treated and untreated waters. For sampling 20ℓ of drinking water, 4 foam plugs - two each in two different columns - should be used. The same number of foam plugs should be used for 10ℓ raw water. The water should be heated to 60-65°C prior to passage through the foam column, and flow rate should be maintained at nearly 250 ml/min. Foam plugs following sampling should be shipped in ice to prevent loss of benzo(a)pyrene.

The increase in the efficiency of retention of BaP by heating of water appears to be linked to the desorption of benzo(a)pyrene from suspended particles in water, and to a possible change in the conformation of the polymer at higher temperatures. It is felt that the retention of other PAH as well as other compounds, e.g. PCB's, pesticides, on polyurethane foam will also be enhanced if water temperature is increased, and should be investigated.

SECTION VI.

METHOD OF PAH ANALYSIS

BASIS FOR DEVELOPING ANALYTICAL METHOD

The previous section describes the preconcentration of BaP from water samples using polyurethane foam plugs as sorbent. The use of radiolabelled-BaP eliminated the need for any chemical separation procedure for its isolation from impurities and the need for any selective identification method for its estimation. Establishing the general validity of the method towards other PAH requires the determination of the efficiencies of retention of other PAH and thus the development of an analytical procedure involving clean-up of foam extract and a selective identification procedure for their quantitation. The analytical procedure is required to provide separation to the extent necessary for their interference-free detection. The problem in the development of an analytical method for the determination of PAH which usually occur as a small quantity in a large matrix of impurities is three-fold. First, the compounds of interest must be adequately separated from impurities concentrated from water along with PAH and those which are eluted from foam, second, the PAH must be separated from each other, and third, a high sensitivity of detection is required to quantitate small amounts of PAH.

The choice of procedure for the separation of PAH from the interfering classes of compounds depend largely on the type of sample to be analyzed. Although the literature concerning PAH separation in various other types of samples is abundant (8,13,14,28), it is not so in the case of treated and raw waters. Irrespective of the nature of sample, no single analytical separation procedure to date is capable of providing complete separation and resolution of the PAH fractions. Therefore, analyses of PAH mixtures have typically entailed various partitioning sequences followed by column, thin layer or paper chromatography prior to detection.

Acheson *et al.* (1) reported that TLC procedure for separation of PAH compounds was highly effective and less susceptible to interference by background organic materials. TLC has been successfully used for separation of PAH isomers (40). In recent years, a number of researchers have used TLC in combination with other methods for the separation and identification of PAH in complex mixtures (9,26).

Gas chromatographic separation of PAH compounds using Dexsil-300 (18,27) column is another versatile method which combines simplicity of operation with relatively high sensitivity. Strosher and Hodgson (48) have separated PAH compounds from lake waters and associated sediments using this column.

However, the resolution of some of the closely related compounds has not been achieved. Janini et al. (24) have claimed that a new G.C. column using nematic liquid crystal can accomplish the above separation. But column bleed at higher temperatures still remains a problem with this packing material.

Although surface coated, open tubular (SCOT) columns (27,28) and high efficiency glass capillary columns (30) have been used in the past for separation of PAH, their limited total sample load capability restricts the detection limit to an undesirable value. High pressure liquid chromatography (hplc) with bonded octadecylsilyl phases of microparticle size (16) is a particularly promising technique for the separation of PAH compounds. But the cost of the instrumentation makes it a restrictive alternative to other techniques available for routine analysis.

With regard to detection, fluorescence spectroscopy has become well established as a sensitive and selective analytical technique for PAH. Several groups (12,46) have found it to be at least ten times more sensitive than U.V. method. It is also more sensitive and less expensive than mass spectral detectors. Mass spectrometers normally have a nanogram detection limit (21,41), although integrated ion current techniques reduce this limit to the subpicogram range (33,39).

This section describes a method for the separation of PAH from impurities, and their qualitative and quantitative determination. The clean-up procedure utilizes the ability of the PAH compounds to form charge-transfer complexes for separating these compounds from the bulk of impurities. Further purification which was found necessary was performed on a short Florisil column. The identification and quantitation was done by GLC-flame ionization techniques, and by spectrofluorophotometry following two dimensional thin layer chromatography.

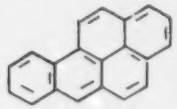
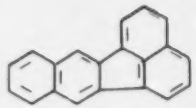
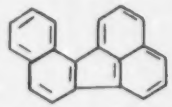
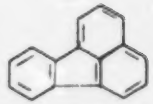
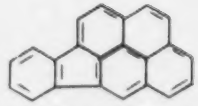
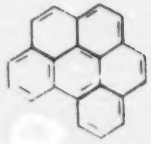
SELECTION OF PAH FOR STUDY

The number of PAH which have been detected in environmental samples is considerable. Since it is impossible to detect all of them it was found practical to choose several specific and representative compounds and limit the monitoring phase of the project to these.

The six PAH included in this study are: benzo(a)pyrene, fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, indeno(1,2,3-cd)pyrene, benzo(ghi)-perylene. With the exception of benzo(j)fluoranthene, WHO (5) recommends analysis of these PAH in drinking waters. Benzo(b)fluoranthene, which is recommended for analysis by WHO, has been replaced with benzo(j)fluoranthene in our analysis due to its non-availability. The group of the 6 PAH is regarded as representative of the whole polynuclear family.

The structure and carcinogenic properties of the selected PAH are given in Table 9.

TABLE 9. PAH COMPOUNDS STUDIED

Symbol	Compound	Synonym	Structure	Emperical formula	Carcinogenic Potency*
BaP	benzo(a)pyrene	3,4 benzpyrene		$C_{20}H_{20}$	+++
BkF	benzo(k)fluoranthene	8,9-benzfluoranthene		$C_{20}H_{12}$	-
BjF	benzo(j)fluoranthene	7,8-benzfluoranthene		$C_{20}H_{12}$	++
FL	fluoranthene	--		$C_{16}H_{10}$	-
IP	indeno(1,2,3-cd)pyrene	0-phenylenepyrene		$C_{22}H_{12}$	+
BPR	benzo(ghi)perylene	1,12-benzperylene		$C_{22}H_{12}$	-

* National Academy of Sciences, 1972 (36)

EXPERIMENTAL METHODS AND RESULTS

Necessary Precautions

In the analysis of samples containing PAH in ppb-ppt range, the necessity of preparation of scrupulously clean glassware cannot be overemphasized. All the glassware should be free from grease, and Teflon stopcock should be used in place of glass. No smoking should be permitted in the laboratory. PAH are light sensitive and therefore all the glassware used in transportation, analysis and storage of PAH must be wrapped with aluminum foil and all the work be done in subdued light. Appropriate precautions must be taken in working with these compounds because of their carcinogenic nature.

Materials and Reagents

PAH Standards: Fluoranthene, Benzo(ghi)perylene, Indeno-(1,2,3-cd)pyrene and Benzo(a)pyrene were obtained from Aldrich Chemical Co.; Benzo(j)- and (k) fluoranthene were supplied by Mr. J.L. Monkman, Air Pollution Control Directorate, Ottawa, Canada.

Materials for gas liquid chromatographic analysis:
Gases for GLC: N_2 and H_2, prepurified grade; air breathing grade: Linde Division, Union Carbide Corp.

Aluminum foil backed septa (Metasep), GLC 6 ft x 1/8 inch matched columns packed with 3% Dexsil 300 on chromosorb W (A.W.) 100/120 mesh from Altech Associates.

GLC with dual flame ionization detector: Hewlett-Packard, Model No. 5730A.

Materials for thin layer chromatography:
Acetylated cellulose (40%), Aluminum Oxide G, type E, 8 x 8 cm. glass plates, TLC Tank, Desaga template, Desaga Brinkman spreader: Brinkman Instrument, Inc. Spectrophoto-fluorimeter with thin film scanner; American Instrument Co.

U.V. lamp (Mineralight) for visualization of TLC spots: Scientific Products, Inc.

Other Materials:

Solvents, Distilled in glass: Mallinckrodt Chemical Co. and Burdick Jackson Lab, Inc.

Florisil (60-100 Mesh) chromatographic grade, Matheson Coleman and Bell, Inc.

Rotary vacuum evaporator with thermoregulator and immersion heater, Buchler Model, Scientific Product, Inc.

Calibrated tubes (6.5 ml): Fisher Scientific Co. Each of the last four tenth ml is calibrated to one-hundreth ml.

Source of other materials and reagents not described here - see Section V., p. 8.

Standard PAH Solution

Stock solutions of each of the six PAH are prepared by dissolving 100 mg in 100 ml benzene in a volumetric flask (1000 ppm).

In order to prepare standard PAH mixture for gas chromatographic analysis, mix 1 ml each of the six stock solutions in a 10 ml volumetric flask and make up the volume with benzene (final concn. 100 ppm). Standard PAH mixture for TLC is prepared by mixing 5 ml of the standard fluoranthene solution and 1 ml each of the five other PAH in a glass stoppered bottle (final concn. 50 ppm fluoranthene, 10 ppm others).

PAH Separation and Analysis

PAH analysis was performed using two techniques - gas liquid chromatography - FID detection, and thin layer chromatography-fluorescence detection. The relative merits of these and other methods of PAH analysis have been discussed by many researchers (1,44). The TLC procedure used was of Borneff (6) and recommended by the World Health Organization (55). The method was employed by us after slight modifications. It permits qualitative as well as quantitative determination of PAH directly on the TLC plate, and thus losses associated with removal of PAH for fluorescence measurement are eliminated.

The details of the two methods and their sensitivity and scope under our experimental conditions are given below.

Gas Liquid Chromatography - FID Detection--

The gas chromatograph used was equipped with a dual flame ionization detector and a linear temperature programmer. The experimental conditions used were as follows:

Column: 6 ft x 1/8" stainless steel packed with 3% Dexsil 300 on chromosorb W, 100-120 mesh (columns preconditioned at 325°C for 24 hrs. and operated in differential mode)

Carrier gas (N_2) flow rate: 30 ml/min.

H_2 gas flow rate: 30 ml/min.

Air flow rate: 300 ml/min.

Detector temp.: 300°C

Injection Port temp.: 250°C

Column oven temp. programming:

Initial temp.:	200°C
Initial delay:	2 min.
Program rate:	4°C/min.
Final temp.:	290°C
Final delay:	8 min.

The maximum temperature of 290°C used in the present study for GLC temperature programming completely eluted all the six components. This is in conformity with the work of Lao et al. (27). The temperature programming, however, caused a stepwise increase in the baseline up to the maximum programmed temperature and then the baseline fell rather sharply during the cooling cycle. To compensate for the column bleeding effect, an identical second column was used in a differential mode. Even under this condition, the bleeding from septa remained as a problem. With most septa used, at least seven ghost peaks were evident in the chromatograms. The use of aluminum-foil backed septa (Metasep) which had been preconditioned for 12 hours at 250°C gave satisfactory results.

To avoid the error due to dead volume in the syringe, the following solvent flush injection technique was used:

Draw about one µl of solvent into the syringe and withdraw the plunger to introduce an air gap of about 1 µl. Insert the syringe needle into the solution to be analyzed and withdraw the plunger till the top of the air gap differentially moves to the desired volume to be sampled. Withdraw the syringe needle from the solution and withdraw the plunger till an air gap shows at the bottom of the barrel. Precisely read the volume of the liquid trapped between the two air gaps (see diagram below).

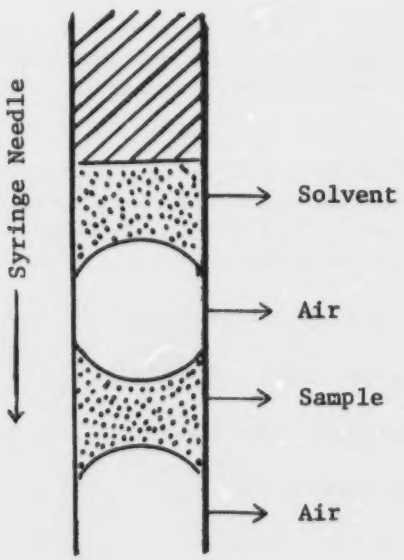


Fig. 6 illustrates the gas chromatograms of the standard PAH mixture. The identification of the PAH on the chromatogram was achieved by comparison of the relative retention times (RRT) of each PAH determined under identical conditions. The 6 PAH injected resulted in 5 peaks, the isomers - benzo(k)-fluoranthene and benzo(j)fluoranthene - could not be separated on this column.

Quantitative estimation of the individual PAH concentration was performed by peak height measurement for the peaks which were sharp and symmetrical. PAH showing wide peaks were quantitated by evaluating their area with the method of multiplication of the peak height by the width at half height. Standard curves for each compound were constructed to find out the linearity of response with concentration. Fig. 7 shows two such curves obtained from fluoranthene and benzo(ghi)perylene, the first and last eluent from the GLC column.

As revealed earlier, the gas chromatographic column used was unable to separate the isomers benzo(j)- and benzo(k)fluoranthene. From the relative retention times compiled by Lao et al. (27) for 12 ft x 1/8" Dexsil 300 column it can be predicted that present GLC conditions will not separate the following groups of compounds: benzo(b)-, (j)- and (k)-fluoranthene; benzo(a)- and (e) pyrene/perylene; indeno(1,2,3-cd)pyrene/benzo(b)-chyrsene/picene; and benzo(ghi)perylene/anthranthene. The incomplete resolution will result in higher values for the estimated PAH concentrations in environmental samples than actually present. The other limitation of GLC analysis using liquid injection technique is its inherent limit on the sample volume (<5µl) that can be injected. This makes the detection of low levels of PAH impossible. In view of these disadvantages of the GLC method, efforts were directed to evaluate the scope of the TLC-fluorometric method of PAH analysis.

Thin-layer Chromatography-Fluorescence Detection--

Preparation of Plates: Mix 28g aluminum oxide G, type E, 12g 40% acetylated cellulose and 2g $CaSO_4.2H_2O$ (200 mesh) with 83 ml 95% ethanol with a magnetic stirrer for 5 min. The resultant slurry was spread to a thickness of 250 µm on eight 20 x 20 cm glass plates using the spreader. The plates were dried for 1/2 hour and approximately 2 mm of adsorbent was scraped from all sides to prevent "edge effects." Activate the plates in an oven for 30 minutes at 80°C. Store the plates in a desicator containing silica gel. The adsorbent layer on the plates is extremely fragile and should be handled with special care.

Sample Application: With the help of a syringe, appropriate aliquot of the concentrate or standard was applied on one corner about 2 cm away from both sides of the plate. Samples were applied in successive small doses and dried by passing prepurified grade N_2 to minimize spot size. For best results, the amount of individual PAH should remain between 20-100 ng/spot.

Development of Plates: The first dimensional development of the plates was done in a n-hexane: benzene (4:1) in a tightly sealed development tank. The solvent front was allowed to run about 2 cm below the dry end of the plate. The development time was approximately 30 min. After drying, the plate was rotated by 90° and developed in the second direction with methanol: ether:water (4:4:1). It takes approximately 2 hours for the solvent front to

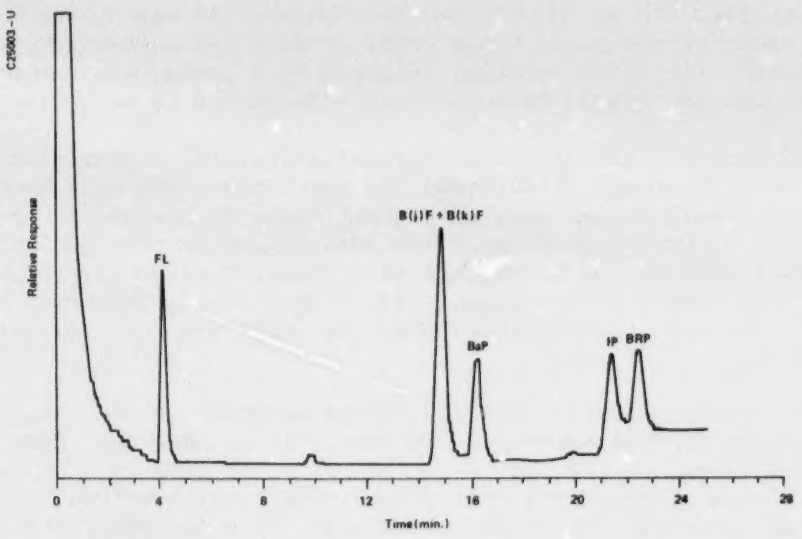
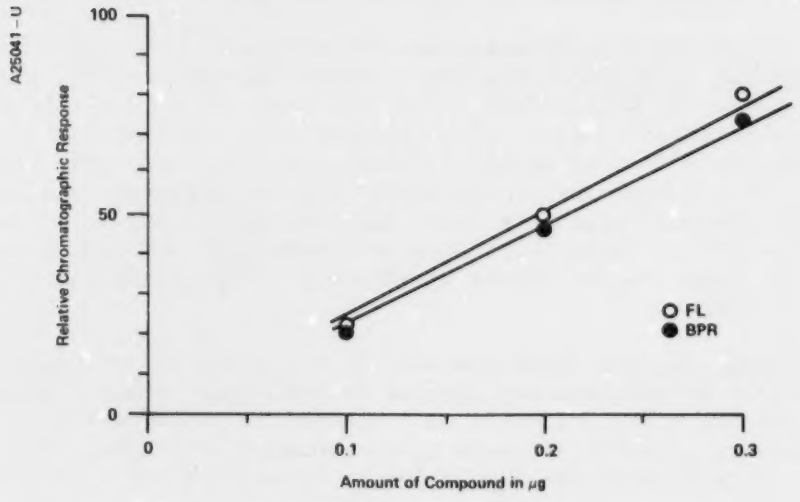


Figure 6. Gas chromatogram of the standard PAH mixture on Dexsil 300 packed column.



Figures 7. Calibration curves for two representative PAH determined by GLC-FID method.

run about 2 cm below the end of the plate. The plates were then air dried and luminescent spots visualized by illuminating the plate in dark with a low intensity U.V. lamp. The boundaries of the spots were marked with a clean, sharp stainless steel needle. If necessary, developed plates can be stored in the dark in a desicator.

The excellent resolution obtained with the TLC system (Fig. 8) for compounds not separated by the GLC method prompted further use of the method. The R_B values defined as the ratio of the distance travelled by a specific compound to that of benzo(a)pyrene appear in Table 10 for the two solvent systems. The values range from 0.5 - 1.8 in the first system and 1 - 3.1 in the second system, showing good separation for the 6 PAH.

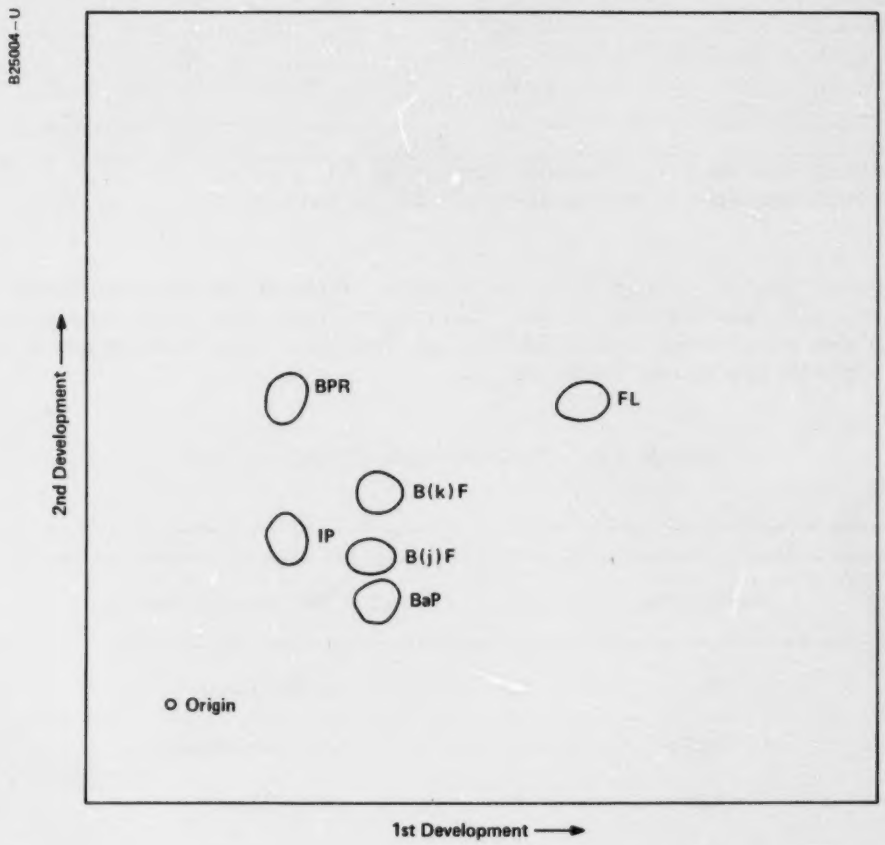


Figure 8. Thin-layer chromatogram of standard PAH mixture (100 ng fluoranthene and 20 ng each of the other 5 PAH).

TABLE 10. R_B VALUES OF PAH IN TWO SOLVENT SYSTEMS

PAH	R_B Value	
	Solvent system 1	Solvent system 2
FL	1.87	3.15
BjF	0.94	1.46
BkF	0.99	2.13
BaP	1.0	1.0
IP	0.60	1.62
BPR	0.57	3.19

Solvent system 1: n-hexane-benzene (4:1)
Solvent system 2: methanol-ether-water (4:4:1)

Identification of Spots on TLC plate: Identification was based on 3 criteria: (i) fluorescence color, (ii) R_B values, and (iii) characteristic bands in the excitation and fluorescence spectra. The fluorescence colors of the six PAH are given in Table 11.

TABLE 11. FLUORESCENCE COLORS OF PAH

Compounds	Color of fluorescence
FL	light blue
BjF	yellow-orange
BkF	dark blue
BaP	violet
IP	yellow-green
BPR	violet

The emission and excitation spectra of all the spots were run directly on the plates with the help of spectrophotofluorometer equipped with a thin-film scanner. The excitation and emission sweep power of the monochromotor was synchronized between 200 and 800 mμ of the X-Y recorder chart paper. The photomultiplier slit was so adjusted that it provided the fine structure in the spectra. Emission spectra of each individual spot except the suspected fluoranthene spot was obtained by setting the excitation wavelength at 300 nm and scanning each spot between 350 and 550 nm. In case of fluoranthene, the excitation wavelength was set at 280 nm and the spot was scanned from 400 to 550 nm. Similarly, the excitation spectra of each spot was obtained by setting the emission wavelengths at one of the emission maxima exhibited in the emission spectra and scanning the spot from 220 to 400 nm. The excitation and emission wavelengths used for running spectra are summarized in Table 12.

TABLE 12. EXCITATION AND EMISSION WAVELENGTHS USED FOR RUNNING SPECTRA

Compound	Emission spectra λ for excitation (nm)	Excitation spectra λ for emission (nm)
FL	280	458
BjF	300	427
BkF	300	428
BaP	300	427
IP	300	467
BPR	300	430

The identification on the basis of fluorescence color is at best tentative. In a complex chromatogram, the identification on the basis of R_B values which may vary by as much as 15% can be misleading (25,43,54). The best method of identification is the matching of the shape and characteristic bands between the unknown and known spots of the standard compounds. Although the emission bands of some of the compounds, e.g., benzo(a)pyrene and benzo(k)-fluoranthene are very similar, their excitation bands are quite distinctive. Similarly, the lack of fine structure in the emission spectra of both fluoranthene and benzo(j)fluoranthene poses no identification problems because of their distinctive excitation spectra. The excitation and fluorescence spectra of the six PAH appear in Figure 9.

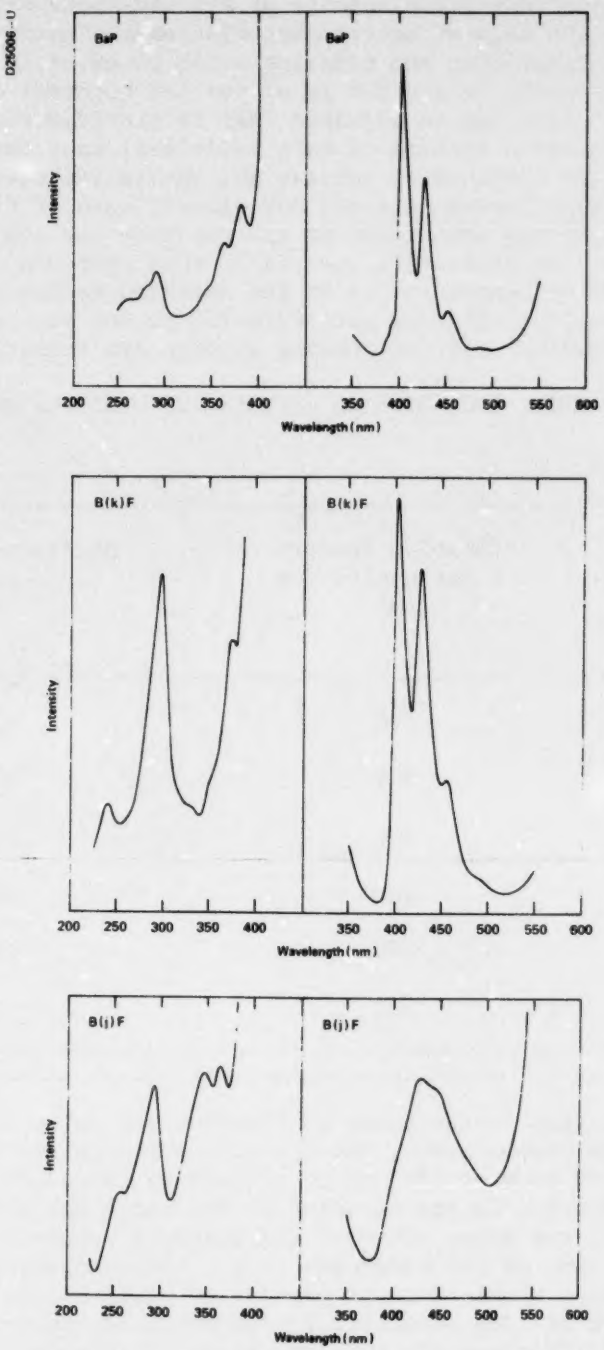
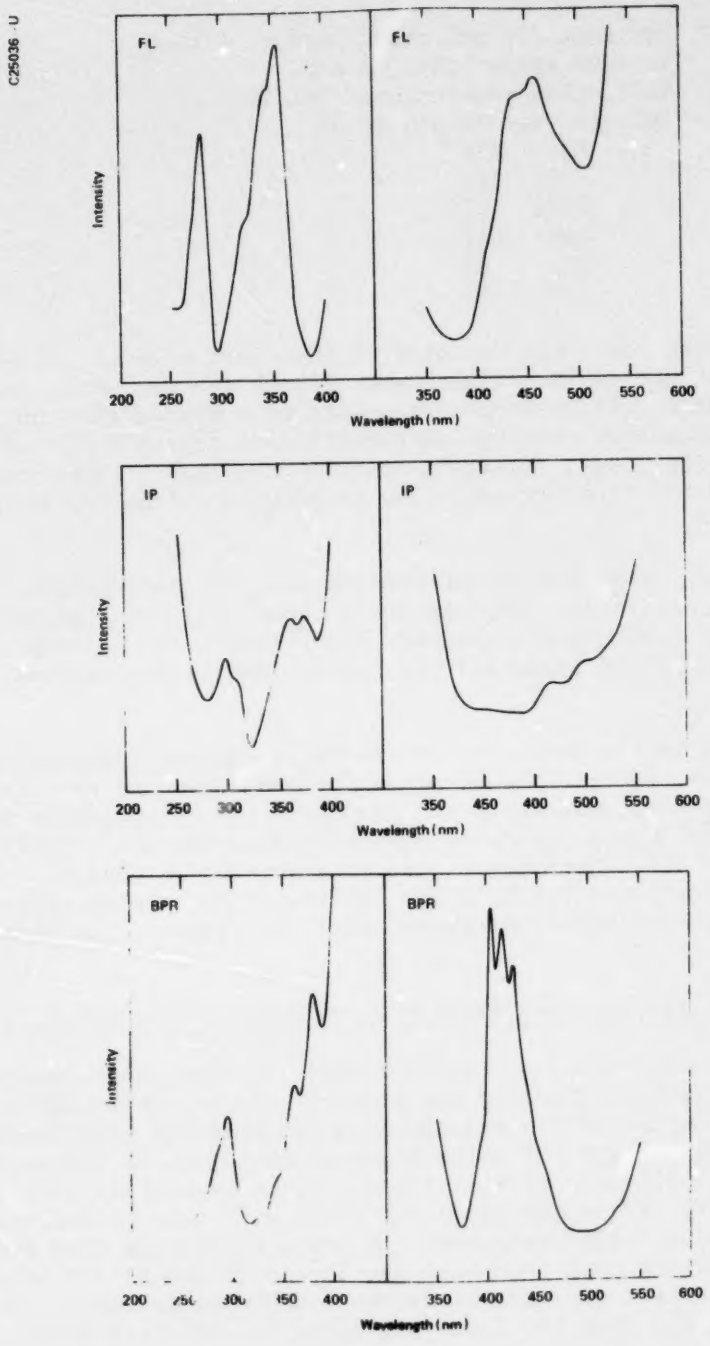


Figure 9. Fluorescence emission and excitation spectra of model PAH compounds obtained directly on the plate.

continued

Figure 9. continued



Quantitative Analysis: Quantitation was performed by scanning each spot directly on the plate for fluorescence intensity. The following experimental conditions were used:

Recorder (50 mv) chart speed: 4"/min.
Scanner cycle time: 2 min.
Excitation wavelength: 365 nm
Emission wavelength in nm
FL: 458
BjF: 427
BkF: 428
BaP: 427
IP: 467
BPR: 416

For each marked spot, the position of the light beam on the spot was adjusted such that the maximum fluorescent signal was recorded. Each spot was, then, scanned for fluorescence intensity at a photomultiplier slit width of 2.0. The output from the photomultiplier tube (IP 28) was applied to a strip chart recorder to obtain a trace of each spot. The direction of scanning was selected in a way such that interference from adjoining spots was minimum.

The concentration of PAH was determined from the calibration curve obtained for each individual compound using three different concentrations. The area under the fluorescence peak was determined with the help of a planimeter. The range of linearity for the six PAH is represented in Figure 10.

The variation in the intensity of the light source causing error in the quantitative values, was studied by measuring the intensity regularly with a standard 100 ng quinine sulfate spot. Any variation of intensity was corrected for in the quantitative values of the PAH concentrations. Studies by Keegan (25) showed that the fluorescence intensity of individual PAH spots decreased significantly as the moisture content of the plates decreased. Therefore, thorough drying of the plates after development was necessary for reproducible results.

Clean-up Procedure for Removing Impurities of Water and Foam Origin

During the concentration of PAH from water on foam plugs, several other contaminants also get concentrated and some get eluted during PAH elution. In addition, several impurities belonging to the foam are also leached during the elution process. Figure 11 shows a gas chromatogram of the water concentrate prepared with the help of foam plugs. These impurities interfered with the analysis of PAH. Pre-cleaning of the plugs with organic solvent by batch or soxhlet extraction failed to remove the trace impurities from foam. In fact, soxhleting of the foam increased the levels of impurities extracted. Efforts were, therefore, directed to devise a clean-up procedure for removing impurities originating from the foams and those derived from water.

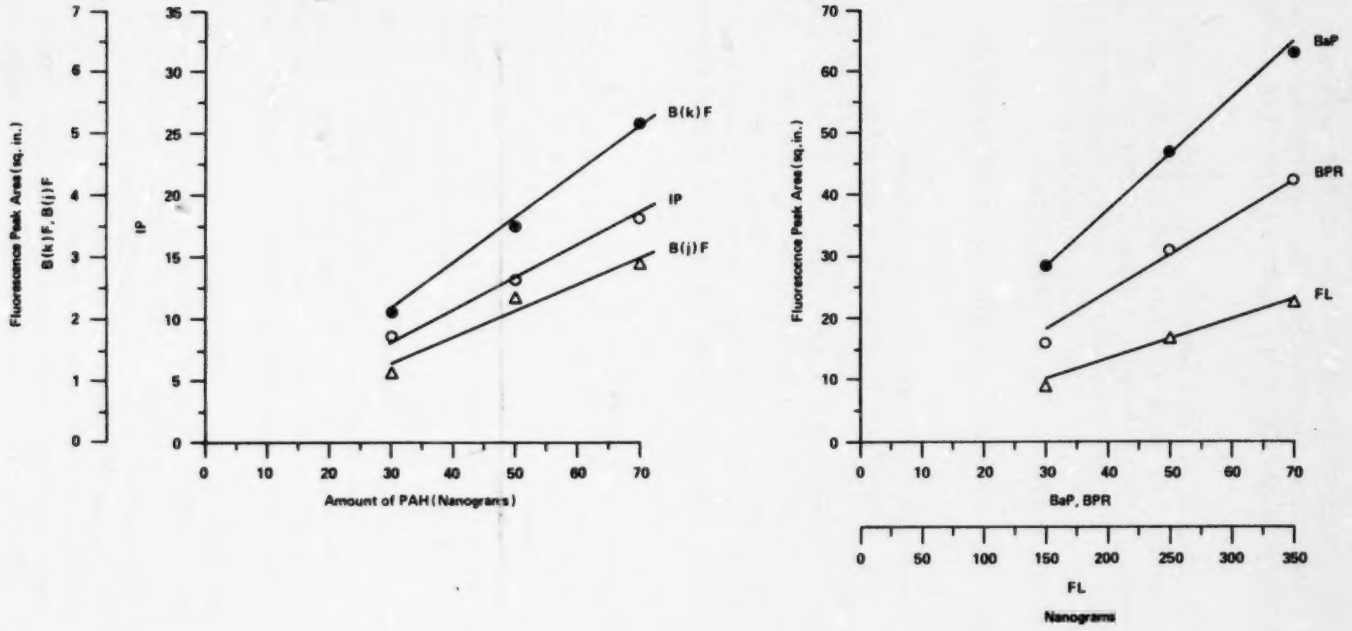


Figure 10. Calibration curves for the six reference compounds determined by fluorimetric method directly on the plate.

The efficiency of the clean-up procedure and the recovery of PAH was first evaluated using gas chromatographic method of PAH analysis. Studies were then undertaken to determine if interferring substances could be detected by thin-layer chromatography which allowed spotting large volumes of the concentrate.

Earlier studies (Section V, p. 21) showed that PAH from 20ℓ of tap water could be effectively concentrated with the help of 4 foam plugs placed in two different columns. Assuming that a sample volume of 60ℓ of tap water should be adequate to detect PAH in drinking waters, efforts were directed to devise a clean-up procedure which will remove interfering substances introduced from 60ℓ of tap water plus those leached from 12 foam plugs.

The details of the clean-up procedure and its efficiency is covered in this section.

Preparation of Sample Containing Interfering Substances--

Twelve foam plugs are placed in 6 Chromaflex columns and washed as described before (Section V, p. 9). Sixty liters of unspiked tap water are passed through the plugs maintaining a temperature at 62 $\pm$ 2°C and flow rate at 250 $\pm$ 10 ml/min. Elute each column with 30 ml acetone and 125 ml of cyclohexane. Mix the eluate and spike it with 10 µg each of the 6 PAH. The contribution of PAH from the tap water passed over the foam plugs was determined to be insignificant in comparison to the amount added and was, therefore, ignored.

Experimental Details of Clean-up Procedure--

(i) Solvent Partitioning - Add 50 ml distilled water to the eluate, shake the contents thoroughly and let it stand till the layers separate. Discard the bottom aqueous/acetone layer and transfer the organic layer with two 20 ml washings of cyclohexane into a round bottom flask. Concentrate the contents to about 10 ml with a rotary evaporator at a temperature of 40°C. Transfer the extract along with two 25 ml cyclohexane washes into a 250 ml separatory funnel. Wash the cyclohexane layer twice with 60 ml 4:1 methanol-water and twice with 60 ml distilled water. Add 20 ml dimethylsulfoxide (DMSO) to the cyclohexane layer and shake the contents for 3 minutes. Let it stand and when the layers separate, withdraw the DMSO layer to another clean 250 ml separatory funnel. Repeat the DMSO extraction two more times and dilute the combined DMSO extract with 120 ml distilled water.

Extract the PAH from the aqueous DMSO phase by shaking it with 40 ml cyclohexane for 5 minutes. Repeat the cyclohexane extraction one more time. If emulsion formation poses any problem at this stage, addition of approximately 0.5 g of cyclohexane washed anhydrous Na_2SO_4 should help breaking the emulsion. Wash the combined cyclohexane layers twice with 60 ml distilled water and dehydrate by passing it through an approximately 15 g Na_2SO_4 bed supported on glass wool and prewashed with cyclohexane. Wash the separatory funnel twice with 10 ml cyclohexane and add the washings to the Na_2SO_4 bed. Wash the Na_2SO_4 bed with an additional 20 ml cyclohexane and collect the cyclohexane and the washings in a 200 ml round bottom flask. Concentrate the extract to 5 ml by rotary evaporation. At no time the PAH mixture should be allowed to proceed to complete dryness. This has been shown to result in a loss of PAH (14).

(ii) Column Chromatographic Clean-up - Further purification of the extract is achieved by column chromatography on a Florisil column. Make a slurry of 8 g of preactivated Florisil in methanol and transfer it into a 1.5 cm i.d. and 30 cm length glass column fitted with teflon stopper. Wash the bed with 100 ml methanol and 100 ml 1:1 hexane-benzene mixture. Activate the column (without the teflon stopper) by placing it in an oven for at least four hours at 130°C. Following activation, cool the Florisil bed to room temperature and wash it with 100 ml benzene. Transfer the PAH containing cyclohexane layer on the Florisil bed with the help of a Pasteur pipet. Wash the round bottom flask thrice with 5 ml benzene and add the washings to the Florisil column. Elute the PAH from the bed with 125 ml benzene at a flow rate of 5 ml/min. and receive the eluate in a 200 ml round bottom flask. Concentrate the eluate to about 2-3 ml by rotary evaporation and transfer the contents with adequate washings to a calibrated tube. The benzene layer is further concentrated to 0.1 ml by passing purified nitrogen and subjected to quantitation.

Results and Discussion--

During the development of the clean-up method, considerable effort was devoted to justify the necessity and verify the reliability of each step. The extraction and partition steps which precede the chromatographic purification of PAH, have been described by other authors (38) for different kinds of material and appear largely empirical. With high resolution gas chromatograph, Novotny, et al. (38) have shown that the chromatogram obtained from airborne particulate samples were vastly dominated by alkane. Since water samples should bear some resemblence with airborne particulate samples, a selective enrichment of the PAH fraction was deemed necessary for water samples as well. Since PAH are known to form charge-transfer complexes with suitable compounds, DMSO was elected to obtain an aromatic hydrocarbon enriched mixture by this method. The partition co-efficient for PAH between cyclohexane and DMSO is high compared to other partitioning agents (19). The isolation of the extracted PAH by dilution with water and back extraction into cyclohexane prevented subsequent evaporation losses due both to volatilization and thermal degradation. These characteristics render DMSO especially attractive. Acheson et al. (1) have shown that DMSO extraction efficiency for the six PAH varies between 90-100%.

The chromatogram of the concentrate purified by solvent partitioning alone is shown in Figure 11. It is evident that several impurity peaks are present suggesting the necessity of further purification of the extract. Column chromatographic purification involving "classical" adsorbents, such as, silica and alumina have several disadvantages - (i) their adsorptivity often resulting in losses of trace constituents and (ii) desorption properties depending strongly on the amount of moisture in the column. Both these factors cause irreproducible results. Gel permeation chromatography (38), on the other hand, is a very time consuming process and is usually used for fractionation of the PAH components in addition to their purification. Such fractionation was found unnecessary in the present method. Chromatography on a short Florisil column was tried for further purification of the PAH. A remarkable visually observable clean-up of the sample is effected in this step. As in all column chromatographic purification techniques, the selection of proper eluting solvent and the volume of the eluent is important to attain

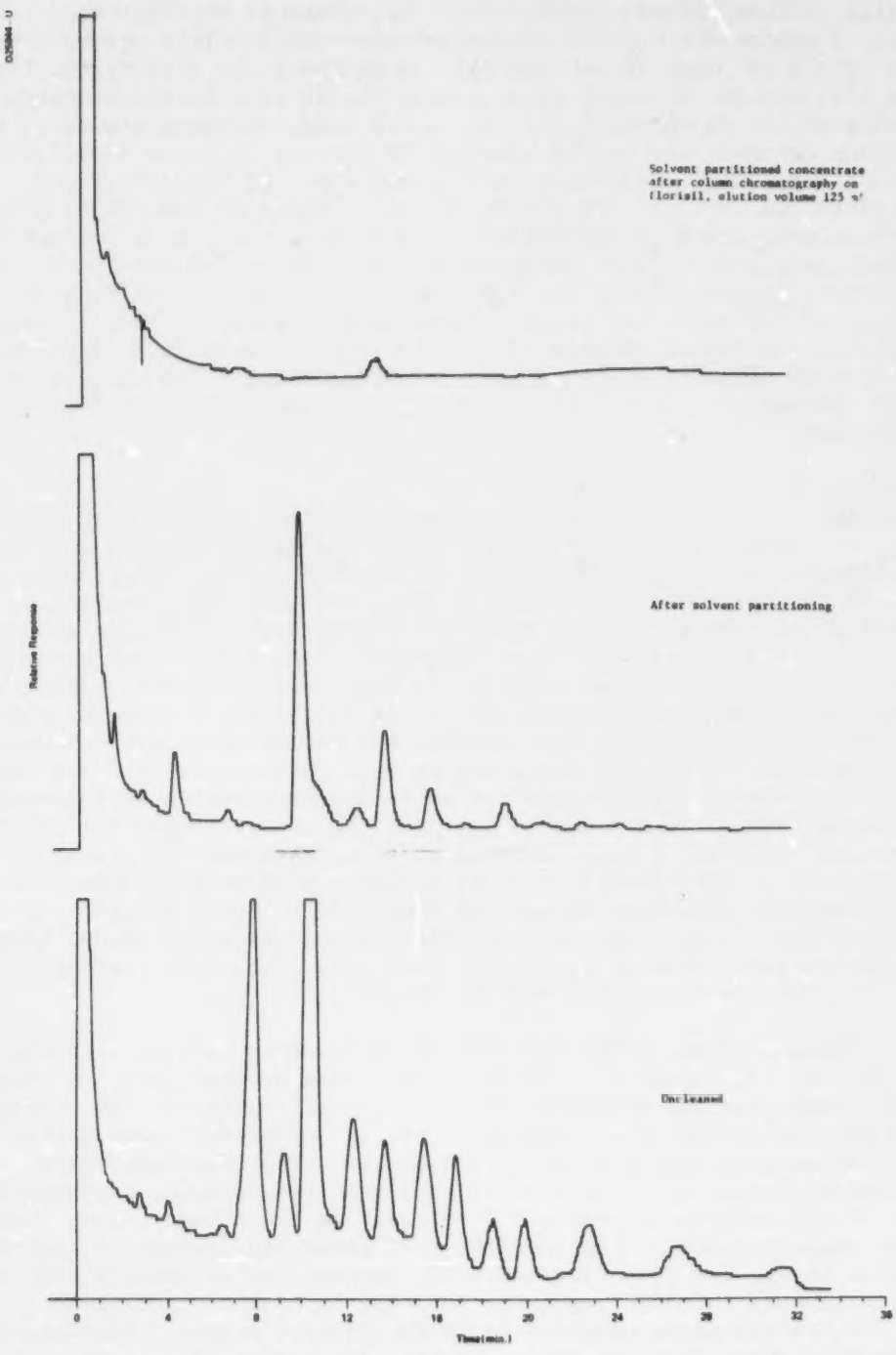


Figure 11. Purification efficiency of the clean-up procedure. Precleaned foam plugs were exposed to drinking water, eluted with organic solvent, eluate concentrated and subjected to GLC.

desirable results. It was found that 125 ml benzene was required to elute all the 6 PAH from the Florisil column without eluting background contaminants (Fig. 11). The PAH recoveries from the complete clean-up step were essentially quantitative (Fig. 12, Table 13) and the extract was sufficiently purified to be readily amenable to GLC analysis. An increase in the eluent volume resulted in elution of impurities from the column.

TABLE 13. RECOVERY OF THE OVERALL CLEAN-UP METHOD DETERMINED BY GAS LIQUID CHROMATOGRAPHY

cpd.	amt. of std. added in µg	amt. of std. recovered in µg	% recovery
FL	10.0	8.97	89.7
B(j+k)F	20.0	20.2	101.0
BaP	10.0	10.0	100.0
IP	10.0	8.93	89.3
BPR	10.0	9.15	91.5

The complete clean-up as used in further studies is illustrated by a flow chart in Figure 13.

The clean-up procedure devised was able to eliminate contaminants to the extent that they did not interfere with PAH peaks in gas liquid chromatography. Since the volume of the extract usually subjected to thin layer chromatography is much larger (see Section VIII, p. 52), it appeared likely that the impurities may become visible and interfere with thin layer chromatography. Thus the efficiency of the clean-up procedure was evaluated using thin layer chromatography. The extract containing contaminants of water and foam origin (60ℓ water, 12 foam plugs), was prepared as described before except that it was not spiked with the standard mixture of PAH. The extract was subjected to the clean-up procedure, concentrated to 0.1 ml and subjected to thin layer chromatography. The chromatogram revealed from none to a maximum of 5 fluorescent spots depending upon the volume of the concentrate spotted. TLC of the concentrate prepared from foam plugs without exposure to water showed that 3 spots had originated from foam plugs (Fig. 14). The other spots were identified to be the PAH concentrated from water (not detectable by GLC). The solvents employed in elution and clean-up were not the source of the impurities, since the concentrate prepared from solvents alone failed to show these spots.

The fluorescent impurities eluted from the foam plugs were studied further to determine if they will cause interference with the analysis of

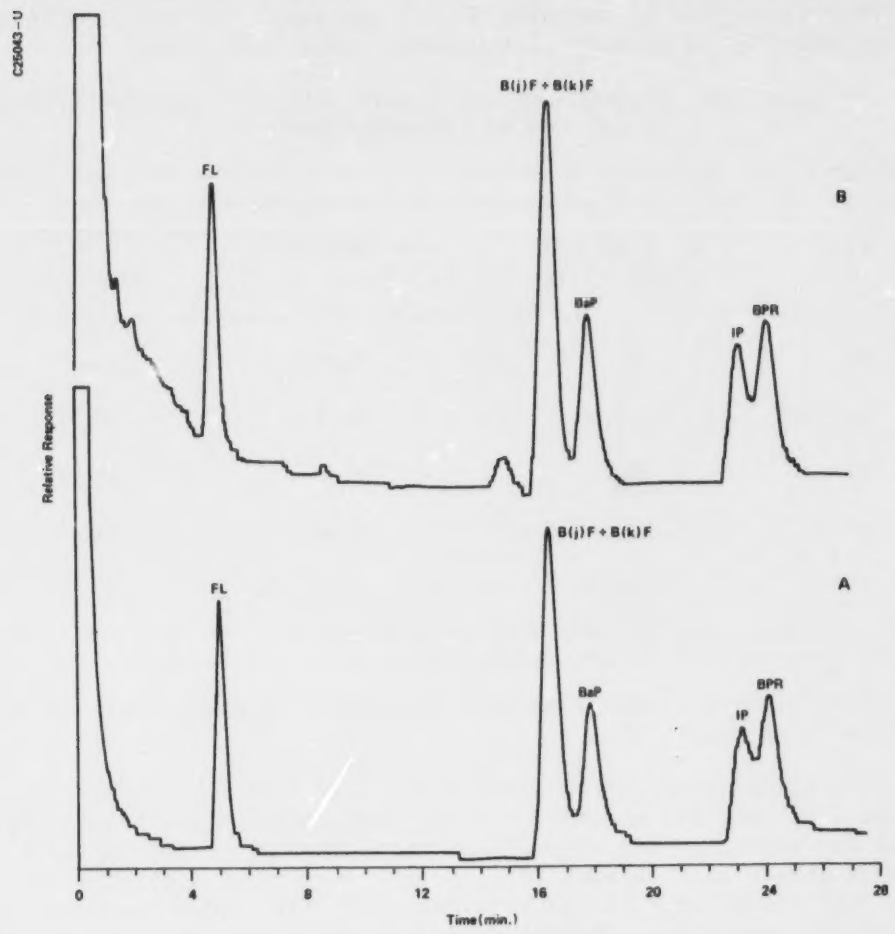


Figure 12. Recovery of PAH in the clean-up process. Precleaned foam plugs following exposure to drinking water were spiked with the PAH mixture. The foam plugs were eluted, eluate concentrated, cleaned up and subjected to gas-liquid chromatography. A. Standard PAH mixture, B. Eluate from water exposed and PAH spiked foam plugs after clean-up.

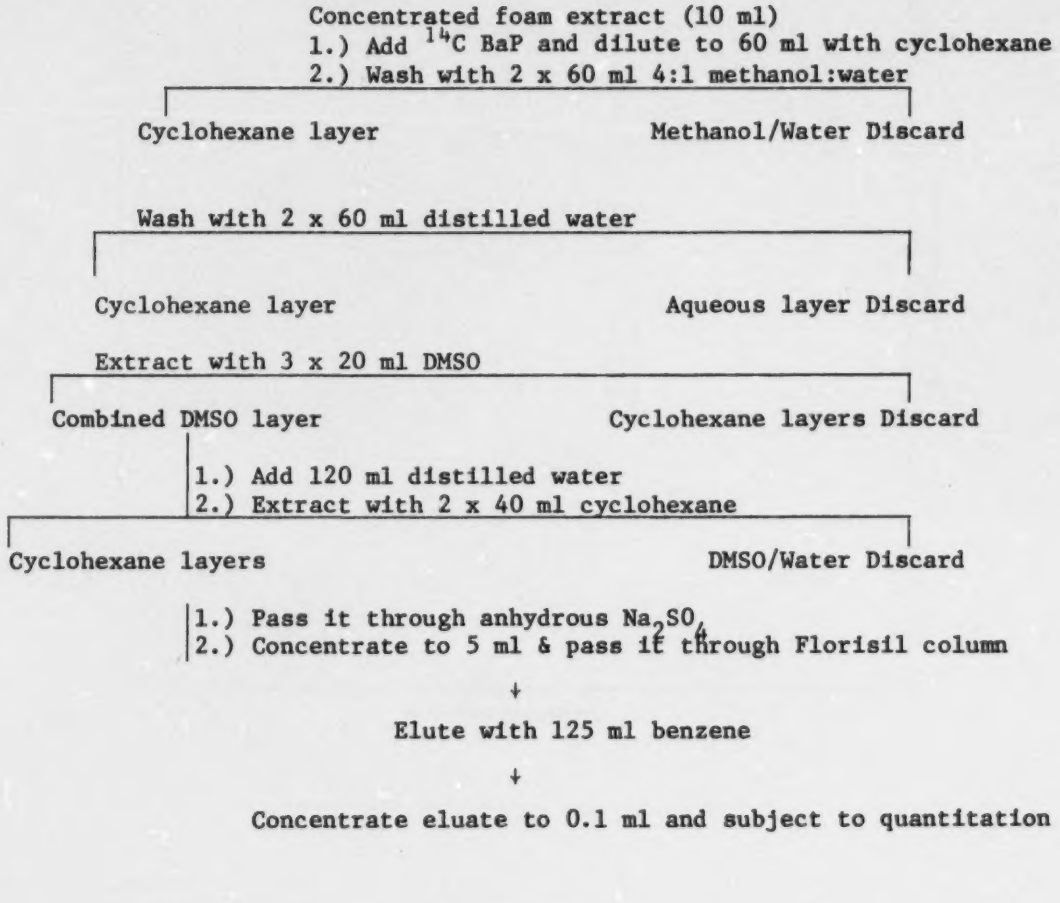

Concentrated foam extract (10 ml)
1.) Add ^{14}C BaP and dilute to 60 ml with cyclohexane
2.) Wash with 2 x 60 ml 4:1 methanol:water
Cyclohexane layer
Methanol/Water Discard
Wash with 2 x 60 ml distilled water
Cyclohexane layer
Aqueous layer Discard
Extract with 3 x 20 ml DMSO
Combined DMSO layer
Cyclohexane layers Discard
1.) Add 120 ml distilled water
2.) Extract with 2 x 40 ml cyclohexane
Cyclohexane layers
DMSO/Water Discard
1.) Pass it through anhydrous Na_2SO_4
2.) Concentrate to 5 ml & pass it through Florisil column
Elute with 125 ml benzene
Concentrate eluate to 0.1 ml and subject to quantitation

Figure 13. Flow chart of the clean-up method.

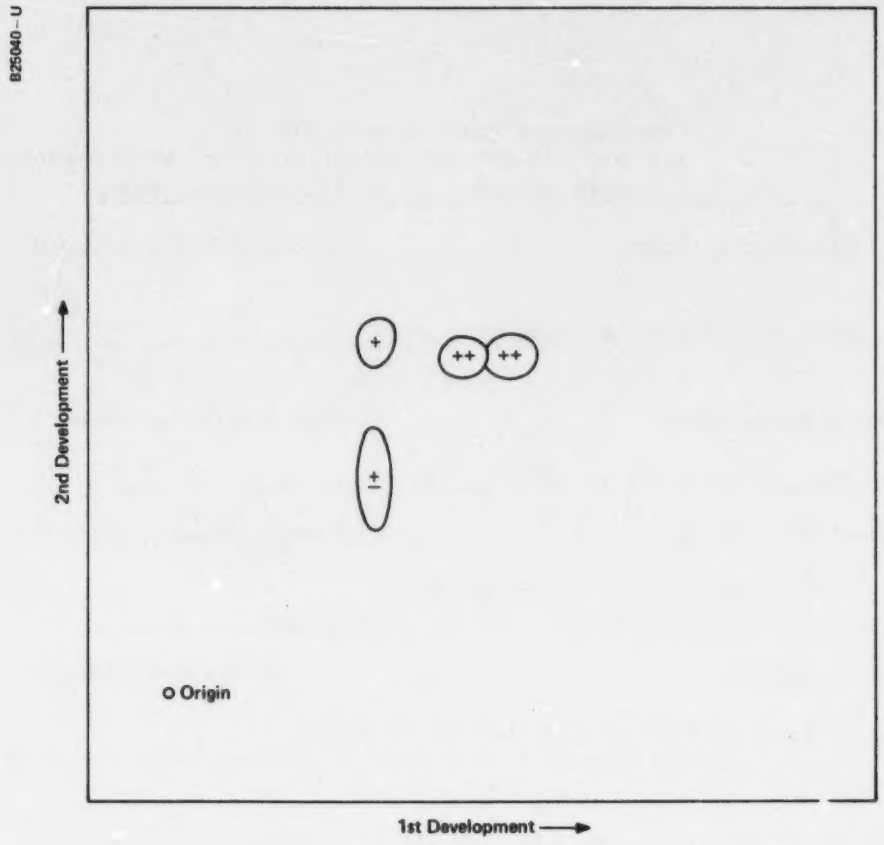


Figure 14. Thin-layer chromatogram of 12 foam blanks. The signs inside the spots indicate relative intensity: ++ moderate, + weak, ± very weak.

6 PAH. This was accomplished by qualitating and quantitating the standard PAH mixture by thin layer chromatography in the presence and absence of the foam extract. Spot 1 (Fig. 14) did not interfere with any of the 6 PAH spots. Of the other two spots, spot 2 remained unresolved with benzo(ghi)perylene and spot 3 with fluoranthene. The fluorescence emission and excitation spectra revealed that spot 2 was benzo(ghi)perylene. Similar studies of spot 3 revealed that it was a composite of an unidentified compound superimposed on a spot recognized as fluoranthene.

The presence of trace amounts of PAH in foam plugs necessitated running a foam blank with each batch of foam plugs to determine the background levels of fluoranthene and benzo(ghi)perylene and correcting the values of these PAH detected in water. The levels of PAH in the foam plugs used for the present study are as follows:

PAH	Total amount (ng) detected in the concentrate prepared from 12 foam plugs
BPR	10.0
FL	70.0

The unidentified compound found close to the fluoranthene spot exhibited its emission minima at the fluoranthene emission maxima and thus presented no problem in quantitation of fluoranthene.

Overall PAH Detection Limit of the Method

Table 14 shows the detection limit for the 6 PAH compounds by gas liquid- and thin layer chromatography. The detection limit for fluoranthene and benzo(ghi)perylene by TLC is restricted by the background level of PAH introduced from the foam plugs. Assuming that the detection limit is twice the background level, the detection limit of these PAH has been derived. The detection limit for the GLC method is based on a minimum output response of five times the background values obtained at an output attenuation of 2 x 10 and a maximum sample loading volume of 5 µl from a total of 100 µl concentrate.

TABLE 14. DETECTION LIMIT FOR SIX PAH BY THIN LAYER- AND GAS CHROMATOGRAPHY

	TLC-fluorometric Detection		GLC-FID Detection	
PAH	absolute limit (ng)	limit in 60ℓ water (ppt)	absolute limit (ng)	limit in 60ℓ water (ppt)
FL	140	2.3	13.6	4.5
BjF	7.5	0.1	10.1	3.4
BkF	5.0	0.1		
BaP	10.0	0.2	11.9	4.0
IP	10.0	0.2	14.7	4.9
BPR	20.0	0.3	14.9	5.0

SECTION VII

EVALUATION OF FOAM PLUGS FOR COLLECTION EFFICIENCIES OF SIX PAH

It is demonstrated in Section V that polyurethane foam plugs effectively retain trace quantities of benzo(a)pyrene from water. The extension of the method to other representatives of the polynuclear family requires determination of the collection efficiencies of those PAH. This section deals with the procedure and results concerning the collection efficiencies of six individual PAH from a representative treated water, and a raw water representing the worst case of raw drinking water source.

Initially, the collection efficiency of the foam plugs was evaluated at PAH concentrations which could be detected by GLC. Experiments were later conducted to determine collection efficiency at lower PAH concentrations and concommitantly large sample volumes. The detection of lower PAH concentration required analysis by thin-layer chromatography and fluorometric detection.

COLLECTION EFFICIENCY OF SIX PAH EVALUATED BY GAS-LIQUID CHROMATOGRAPHY

These studies were carried out with 4 liters of tap water which had been spiked with the standard mixture of six PAH to give a concentration of 25 ppb for each PAH. The water was drawn over a prewashed foam plug maintaining water temperature at 62 $\pm$ 2°C and flow rate at 250 $\pm$ 10 ml/min. PAH were eluted from the foam plug using the procedure described earlier (Section V, p. 10). In order to account for the PAH adsorbed to the walls of the reservoir, the reservoir was washed with benzene-acetone and the washings were combined with the foam eluate. The combined extract was concentrated and subjected to gas-liquid chromatography. Purification of the concentrate was found to be unnecessary because of the presence of high concentrations of PAH and low levels of impurities. The background levels of PAH in the tap water were non-detectable by gas chromatographic method and thus did not affect the results.

Gas chromatogram of the PAH added to water and those recovered from the combined foam and bottle extract are shown in Figure 15. Collection efficiency for each PAH is calculated by comparing the two peak areas. The results shown in Table 15, confirm that polyurethane foam plugs under suitable conditions not only effectively concentrate benzo(a)pyrene but other PAH as well.

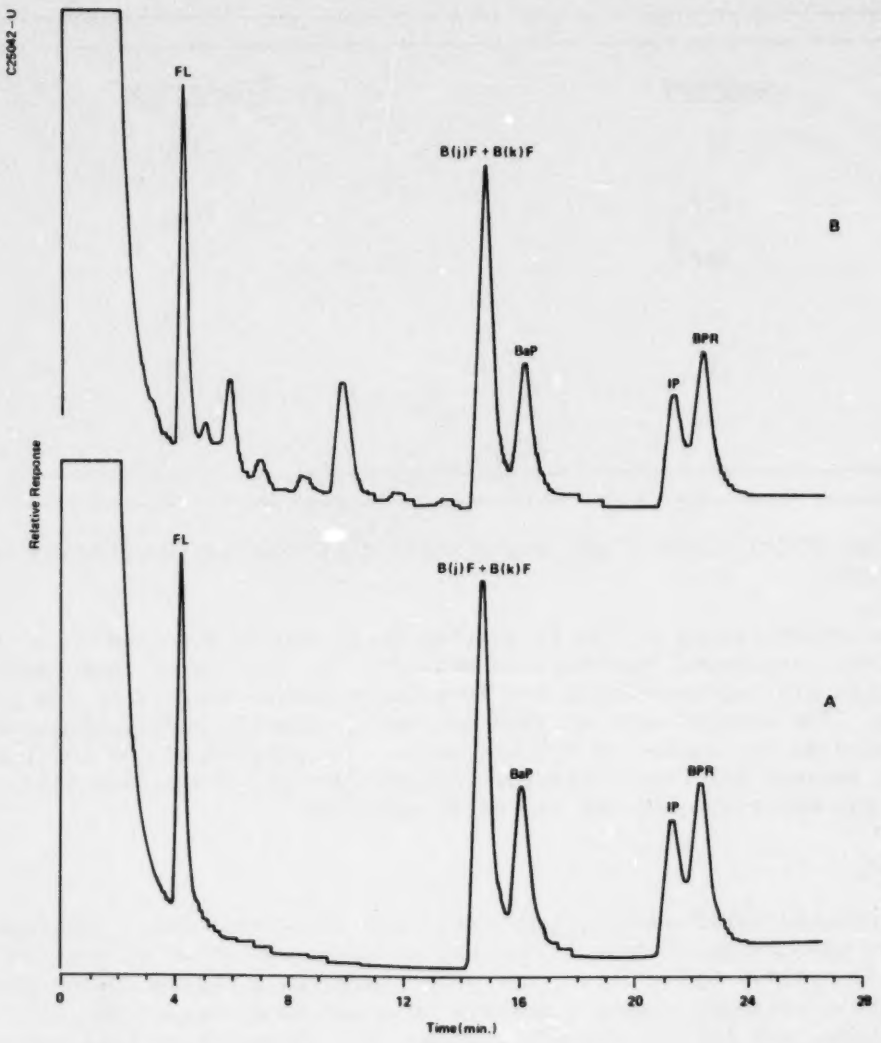


Figure 15. Retention of 6 PAH on polyurethane foam determined by gas chromatography. A. PAH added to water; B. PAH retained on foam plugs.

TABLE 15: FOAM RETENTION EFFICIENCIES OF SIX PAH FROM TREATED WATER. WATER SOURCE: LABORATORY TAP WATER; VOLUME: 4ℓ; CONCN. OF EACH PAH: 25 ppb

Compound	% Retention
FL	100
BjF, BkF	88
BaP	81
IP	89
BPR	91

COLLECTION EFFICIENCIES OF SIX PAH EVALUATED BY THIN-LAYER CHROMATOGRAPHY-FLUOROMETRY

The concentration of PAH in treated water can be expected to be low and thus it was considered important to evaluate the ability of foam plugs to concentrate six representative PAH from large sample volumes at low concentrations. The studies were carried out using thin-layer chromatography-fluorometry as the method of PAH analysis. A concentration of 100 ppt for each PAH (except for fluoranthene which was 500 ppt) and sample size of 60ℓ for treated water and 30ℓ for raw water was used.

Procedure

As treated water source, laboratory tap water was used. Onondaga Lake water was used as an example of a worst possible raw water source (see Section V, p.21). Spike tap or raw water taken in a twelve gallon pyrex glass bottle with PAH standard mixture to a concentration of 500 ppt for fluoranthene, and 100 ppt for all others. Mix the solution well for about an hour with a Teflon magnetic stirrer.

As shown earlier (Section V, p.20), a large portion of the added PAH becomes adsorbed to the walls of the reservoir resulting in a much lower concentration of the individual PAH in the aqueous phase. Since it is impossible to quantitate the loss of PAH by adsorption to 12 gallon glass bottles, the actual concentration of PAH in the aqueous phase was determined by extracting an aliquot of the spiked sample.

Two one-liter aliquots of spiked water were withdrawn, one at the beginning and the other towards the end of the sampling procedure. Each was extracted with 100 ml cyclohexane. The extracts were combined and concentrated to 0.1 ml. Following clean-up, the extracts were subjected to thin-layer chromatography-fluorometric analysis for separation and quantitation of the six PAH. Combining of the two aliquots produced average values of the initial PAH concentration in water.

The spiked water was drawn over four plug system consisting of two precleaned foam plugs in two different columns. In the case of finished water, both columns were changed after every 20ℓ of water, whereas with raw water, the columns were changed after every 10ℓ. Thus, 12 foam columns were required for concentrating PAH from 60ℓ of tap water or 30ℓ of raw water. Foam plugs are then eluted in the usual manner, the eluate concentrated and subjected to the full clean-up procedure, and analyzed by thin-layer chromatography-fluorometry as described earlier.

Results and Discussion

The present method utilizes a direct determination of initial concentration of the PAH in the spiked water phase. This not only takes care of the problem of reduction in concentration due to different kinds of losses, but eliminates the necessity of determination of the background PAH concentrations in the unspiked water samples. The amount of each PAH actually added and that recovered from the aqueous phase is shown in Table 16. The losses of PAH due to adsorption to reservoir surface appear to be somewhat related to the molecular wt. of the compound, for example, in case of BPR (M.W., 274), as much as 77% of the added amount remained adsorbed, whereas only 44% of FL (M.W. 202) was lost due to adsorption. An increase in the adsorption of BaP in these studies compared to the results reported in Section V (p. 20). can be attributed to increase in the size of the reservoir.

TABLE 16. AMOUNT OF 6 PAH UNACCOUNTED FOR AS A RESULT OF MIXING WITH WATER IN A GLASS BOTTLE. WATER VOLUME: 60ℓ, BOTTLE CAPACITY: 12 GALLONS

Compound	Concn. on the basis of amt. added to water (ng/ℓ)	Concn. found in aq. phase (ng/ℓ)	% PAH adsorbed to reservoir surface	Molecular weight
FL	500	278.6	44.3	202
BjF	100	48.3	51.7	252
BkF	100	51.7	48.3	252
BaP	100	36.4	63.6	252
IP	100	25.5	74.5	276
BPR	100	22.6	77.4	274

The retention efficiencies of the 6 PAH from spiked laboratory tap water and Onondaga Lake water are shown respectively in Tables 17 and 18. From both these waters polyurethane foam plugs concentrated PAH almost quantitatively. The efficiency of retention will actually be somewhat higher because

some loss of the PAH sorbed on polyurethane foam plugs occurs during the elution and clean-up (see Section VI, p.41) and such loss has not been corrected for in the data illustrated in the tables.

TABLE 17. FOAM RETENTION EFFICIENCIES OF SIX PAH FROM TREATED WATER, WATER SOURCE: LABORATORY TAP WATER; WATER VOLUME: 60ℓ; CONCN. OF FLUORANTHENE: 500 ppt; ALL OTHERS, 100 ppt

Compound	Concn. present in water (ng/ℓ)	Amt. retained by foam from a liter of water (ng)	% Retention
FL	278.6	260.4	93.5
BjF	48.3	47.4	98.1
BkF	51.7	50.6	97.9
BaP	36.4	33.6	92.3
IP	25.5	23.9	93.7
BPR	22.6	19.8	87.6

TABLE 18. FOAM RETENTION EFFICIENCIES OF SIX PAH FROM RAW WATER. WATER SOURCE: ONONDAGA LAKE; WATER VOLUME: 30ℓ; CONCN. OF FLUORANTHENE: 500 ppt; ALL OTHERS, 100 ppt

Compound	Concn. present in water (ng/ℓ)	Amt. retained by foam from a liter of water (ng)	% Retention
FL	578.1	687.5	118.9
BjF	77.6	94.0	121.1
BkF	66.1	55.6	84.1
BaP	74.5	59.7	80.1
IP	85.2	61.2	71.8
BPR	23.9	28.3	118.4

The retention data given in the tables is for 0.5 ppb for fluoranthene and 0.1 ppb for others. It was not possible to undertake recovery studies with the mixture of 6 PAH at concentrations lower than this for the following reasons. The susceptibility of PAH to be lost by adsorption to reservoir and other surfaces, necessitated determination of the concentration of each PAH in the spiked sample. This involved extraction of PAH from an aliquot of water and their quantitation. At PAH concentration lower than 0.1 ppb, the amount extracted will be difficult to detect accurately. Experiments with radio-labelled BaP described in Section V (p.18) showed that at concentration as low as 2 ppt, foam plugs were able to retain BaP with 87% efficiency. In view of these findings, it is not unlikely that other PAH will also be efficiently retained at such low concentrations.

CONCLUSION

The polyurethane foam retention efficiencies of the 6 PAH from drinking water have been investigated and found to be almost quantitative. Studies conducted with a worst possible raw water for a likely source of drinking water indicate that the retention values for six PAH are quite high. It is concluded that polyurethane foam plugs can be used as a preconcentration method for all of the six PAH from large volumes of both treated and raw water.

SECTION VIII

FIELD MONITORING

The results reported in earlier sections clearly show the potential of polyurethane foam plugs as a convenient method for concentration of PAH from treated and raw water. The next step was to apply this method under field conditions to demonstrate the feasibility of maintaining optimum conditions. Initially, efforts were devoted to fabricate a portable sampling unit which would allow maintaining of temperature and flow rate of water optimum for PAH retention. After evaluation of the unit in the laboratory, sampling of ten drinking water supplies in the Eastern U.S. was undertaken.

FABRICATION OF SAMPLING UNIT FOR FIELD MONITORING

A field sampling equipment was assembled from a combination of custom-made and off-the-shelf hardware available commercially. It consisted of the following five parts as shown in Figure 16: (1) variable water pumping unit, (2) thermostated water circulator, (3) unit containing the column system, (4) temperature monitoring device and (5) flowmeter. The sources of all commercially available hardware are described in Sections V and VI.

The purpose of the pumping unit was to pump water from the sample source through the foam columns at a controlled rate. The unit consisted of two oscillating type pumps connected in series with a minimum amount of thick-walled tygon tubing. The electrical input of the pumps was applied through a Variac. By controlling the output from the Variac, the water pumping rate was controlled at 250 $\pm$ 10 ml/min. Haake thermostated circulator with a custom-made glass coil (25 cm x 6 mm) immersed inside the reservoir housing through the cover plate was used to bring the water to 62 $\pm$ 2°C. One end of the coil was connected to the sampling source and the other end to the column system. The temperature of the water passing through the coil was controlled by means of a 1000W heating element and the thermostating device of the Haake circulator. Two 25 mm i.d. Chromaflex extender type columns were mounted on two column stands which were held on wooden blocks. The columns were connected in series by means of a custom-made double-bent Pyrex tubing to each end of which was attached an adapter. The adapter and Chromaflex columns were connected by means of "O" rings and clamps. For continuously monitoring the temperature of water during passage through the columns, a water trap equipped with a right angle thermometer and an inlet and outlet was introduced in the setup. The inlet was connected to the exit end of the column system and the outlet to the pumping unit. A Gilmont flowmeter calibrated for measuring flow rate of 62°C water was placed at the end of the sampling system.

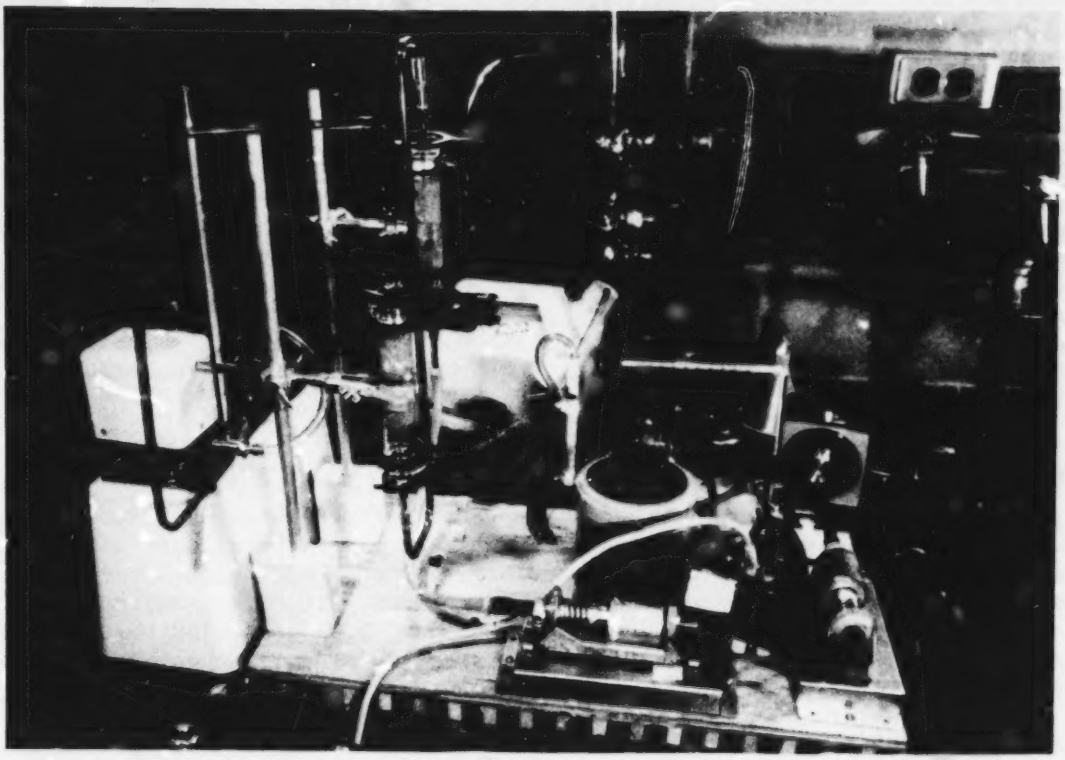


Figure 16. Portable unit assembled for concentrating PAH from water in the field.

The column stands, pumps, Variac, water trap and flow meter were firmly fixed on an 18" x 12" x 1/2" plywood piece as shown in Figure 16. The connection up to the foam columns were made as much as possible with glass tubing and only where necessary with tygon tubing. The type of tubing used for connections beyond this point was immaterial. The overall connection was such that water travelled through the sampling system in the order: sampling source → thermostated circulator → Chromaflex columns → water trap → pumps → flowmeter. This whole unit weighing about 10 lbs. was transported to the sampling site along with the thermostated circulator, an electric timer, a 20ℓ capacity Jerrican and other detachable items, such as Chromaflex columns, thermometers, clamps, "O" rings and connecting tubes. The purpose of the calibrated 20ℓ Jerrican was to collect effluent from the sampler to obtain the volume of water passed through the foam columns. The use of an electric timer for running the unit for appropriate amount of time was optional.

DETERMINATION OF PAH IN SELECTED WATER SUPPLIES

The new method developed was applied to monitoring PAH in ten water supplies in the eastern United States. In some instances, raw intake waters as well as finished waters were examined and the information on the levels and nature of PAH was related to the raw water source, waste/discharges contaminating raw water, and treatment provided. Our sample size was 60ℓ in the case of finished water and 30ℓ in the case of raw water. It was assumed that levels of PAH in raw water will be high and, therefore, concentration of 30ℓ will be adequate.

Selection of Sampling Sites

In selecting sampling locations, several factors which are expected to have an impact on the levels of PAH in finished waters were taken into consideration. These included category of raw water source, waste/discharges entering raw waters, treatment processes provided, etc. The quality of surface waters is dependent on the various sources of pollution that they are subjected to. The selection of sampling sites using surface waters as a raw water source was, therefore, based on the nature of the discharges entering the surface water. Ground water in general is expected to be relatively free of pollution and this prompted inclusion of a water supply using ground water as a raw water source. Several sites were selected which use activated carbon as a treatment process. EPA research (32) indicates that activated carbon removes general organic compounds before attaining its breakthrough. Comparison of the level of PAH in raw and treated water at the above sites, and with the data on the water supply systems which do not use activated carbon treatment, may provide an insight into the effectiveness of the treatment process in removing PAH. Also included in the selected sites are water supplies of 5 major urban centers, each of which serve a large number of consumers.

Treated drinking waters were sampled for all the 10 water supplies but only Pittsburgh, Huntington, Buffalo and Philadelphia were sampled for raw intake waters. Non-availability of the raw intake water source at the other treatment plants forced the exclusion of raw water sampling at these sites. All the monitoring work except the New Orleans sampling was conducted during

the period beginning December 1976 through March 1977, and samples were taken at the treatment distribution sites. New Orleans sampling was done in a motel in the old part of the city in the month of May, 1977.

Table 19 identifies the raw water sources, type of waste/discharge (if any) entering the raw waters, treatment provided and date of sampling for the water supplies selected for this study.

Sampling Procedure

The sampling unit, 6 precleaned foam columns for finished water and 6 for raw water (if applicable) and two empty Chromaflex columns were transported to the sampling site via automobile. The unit was assembled with two empty columns on a counter top or table near the water source. A clean, one liter beaker was placed under the water tap and water from the beaker was pumped through the unit at a flow rate of 250 $\pm$ 10 ml/min. The thermostat in the Haake Circulator was adjusted until the temperature of the flowing water was at 62 $\pm$ 2°C. The setting will vary depending upon the temperature of the water to be sampled and, therefore, it cannot be set in the laboratory. Following this adjustment, the empty Chromaflex columns were replaced with columns packed with foam plugs and 20ℓ of finished water or 10ℓ of raw water was passed over, maintaining the flow rate and temperature as described above. The volume of the water passed was measured by collecting effluent from the sampler in a graduated Jerrican. Both foam columns were changed every 20ℓ in the case of finished water and every 10ℓ in the case of raw water and the sampling was continued to the desired volume of water. The columns were brought to the laboratory for analysis following sampling. No special handling and storage except wrapping of the columns with aluminum foil was necessary if the transit period was less than 2 days, otherwise the wrapped foam columns were cooled with reusable ice packs in styrofoam containers.

PAH Elution and Analysis

The procedure for elution of PAH from foam plugs, clean-up and quantitation has been described in earlier sections. A flow chart of the entire procedure including sampling and shipping is shown in Figure 17.

Addition of Internal Standard and Determination of Recovery Factor

The efficiency of the PAH elution and purification procedure for each analysis was evaluated by addition of an internal standard to the foam plugs upon arrival in the laboratory and prior to initiating any sample work-up. Carbon-14 labeled benzo(a)pyrene was employed as internal standard. A known amount of ^{14}C (approximately 600 dpm) was added to foam plugs, PAH eluted, purified and concentrated as described before. A 10 µl aliquot of the concentrate was assayed for radioactivity in a liquid scintillation system (see Section V, p.10). Knowing the ^{14}C-activity originally added to the foam plugs allows calculation of the recovery factor.

Results and Discussion

The results of analysis of ten treated and four raw water samples are presented in Table 20. No data regarding the precision and accuracy of the

TABLE 19. DETAILS OF THE WATER SUPPLY SYSTEMS USED FOR SAMPLING

Location	Supply System	Water Source	Type of Pollution, if any	Treatment Provided*	Date(s) Sampled
Syracuse, N.Y	City of Syracuse Water Works, Skaneateles, NY	Lake Skaneateles	Uncontaminated lake water	Copper sulfate addition, chlorination and fluoridation	12-16-76
Buffalo, N.Y.	Ward's Pumping Station	Lake Erie	Contaminated with industrial discharge	Coagulation, activated carbon addition, chlorination and fluoridation	12-26/27-76
Pittsburgh, Pa.	Hays Mine and E.H. Aldrich Purification Station	Monongahela River	Contaminated with coke oven effluent	Lime, ferric sulfate addition, activated carbon addition (two stages: (1) powdered carbon[**] (2) granular carbon), chlorination and fluoridation.	1-19-77
Huntington, W. Va.	Huntington Water Corp.	Ohio River	Downstream from coke oven plants	Lime, ferric sulfate and granular carbon addition, chlorination and fluoridation.	1-20-77
Endicott, N.Y.	Endicott Village, Dept. of Public Works	Ground water	Uncontaminated ground water	Chlorination and fluoridation.	2-22-77
Hammondsport, N.Y.	Hammondsport Village, Dept. of Public Works	Keuka Lake	Contaminated with agricultural and winery waste	Chlorination	2-28-77
Philadelphia, Pa.	Torresdale Water Treatment Plant	Delaware River	Contaminated with municipal waste	Ferric chloride, lime, activated carbon ammonia addition, chlorination and fluoridation.	3-5/6-77
New York, N.Y.	Dept. of Water Resources	Croton Reservoir	Uncontaminated upland water	Copper sulfate addition, aeration, corrosion control, chlorination and fluoridation.	3-17-77
Lake George, N.Y.	Lake George Village, Dept. of Public Works	Lake George	Contamination from recreational sources	Chlorination	3-26-77
New Orleans, La.	Tap Water from a Motel in Downtown New Orleans	Mississippi River	Downstream from industries on Mississippi River	Chemical treatment to control alkalinity, hardness and organics; coagulation, ammonia addition, and chlorination.	5-1-77

* Not necessarily in the proper order of treatment, & filtration steps wherever used during the treatment are not shown.
** Used temporarily

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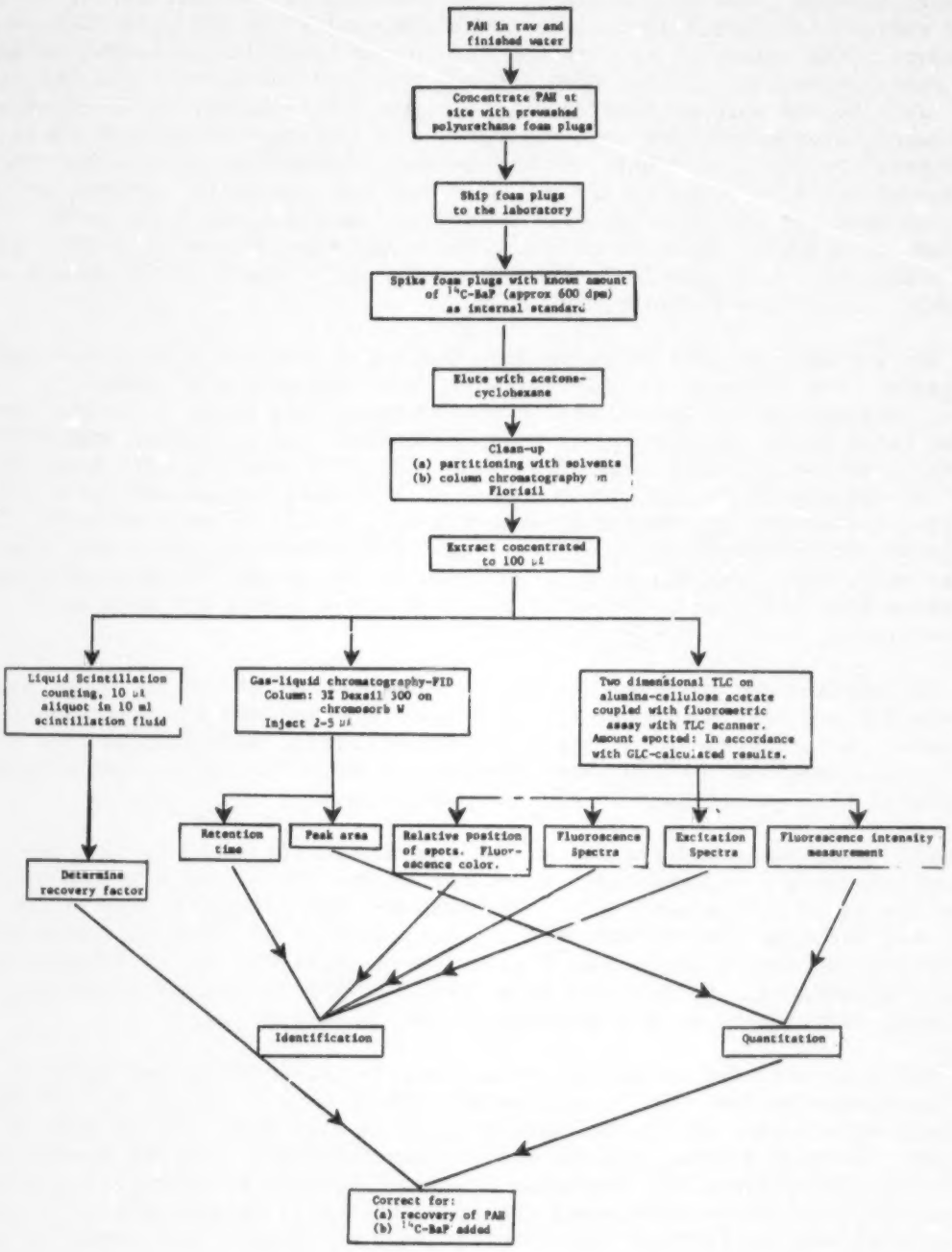
PAH in raw and finished water
Concentrate PAH at site with prewashed polyurethane foam plugs
Ship foam plugs to the laboratory
Spike foam plugs with known amount of ^{14}C-BaP (approx 600 dpm) as internal standard
Elute with acetone-cyclohexane
Clean-up
(a) partitioning with solvents
(b) column chromatography on Florisil
Extract concentrated to 100 µl
Liquid Scintillation counting, 10 µl aliquot in 10 ml scintillation fluid
Gas-liquid chromatography-FID
Column: 3% Dexsil 300 on chromosorb W
Inject 2-5 µl
Two dimensional TLC on alumina-cellulose acetate coupled with fluorometric assay with TLC scanner.
Amount spotted: In accordance with GLC-calculated results.
Retention time
Peak area
Relative position of spots. Fluorescence color.
Fluorescence Spectra
Excitation Spectra
Fluorescence intensity measurement
Determine recovery factor
Identification
Quantitation
Correct for:
(a) recovery of PAH
(b) ^{14}C-BaP added

Figure 17. Flow chart of the method of PAH analysis in water.

results can be given since a single analysis for each sample was performed. Caution must be exercised in making interpretations from these results. The recovery factors given in the tables are derived from the recovery of the added radioactive benzo(a)pyrene internal standard after the full clean-up procedure. The values of the six PAH determined have been corrected using this recovery factor. It has been assumed that the recovery of all the six PAH will be the same as benzo(a)pyrene. The values given for fluoranthene and benzo(ghi)perylene have been corrected for the trace amounts of these PAH contributed by the foam plugs. Benzo(a)pyrene values have been corrected for the amount of ^{14}C-benzo(a)pyrene added as internal standard. Correction has not been made for the retention efficiency of the foams which has been assumed to be 100%. Since in reality, the retention efficiency of foam plugs will always be lower than 100%, the actual amount of PAH in water should be slightly higher than shown in the table.

The BaP used in this investigation has a specific activity of 8.1 mc/millimole. The addition of 600 dpm as internal standard will amount to 8.4 ng increase in the amount of BaP concentrated from water. Since a tenth of the total final concentrate is used for radioactive counting, this will amount to 60 dpm which is equivalent to 48 cpm with counting efficiency of 80%. A substantial change in the amount of ^{14}C added may present problems. Lowering the amount of added radioactivity will result in statistically insignificant difference between the counts determined and background counts. On the other hand, any substantial increase in the amount of radioactivity and hence BaP, might make the detection of the low levels PAH from water inaccurate.

The elution and clean-up efficiency for the treated and raw water samples average 80% and 69%, respectively. The lower average value in the case of raw water is not unexpected since the recovery factor is dependent upon the quality of water samples. However, evaluation of individual recovery factor eliminated this uncertainty in the reported values.

The final concentration of the PAH presented in Table 20 is based exclusively on the TLC-spectrofluorometric value. The GLC-FID method in most cases failed to detect the PAH. Furthermore, the concentrations of PAH in water derived from the GLC values are susceptible to an error due to large multiplication factor since only 2 µl volume is injected out of a total of 100 µl concentrate. In spite of this, whenever the GLC values could be obtained, they served as a crosscheck of the TLC-value.

PAH were detected in the ppt range in both raw-and-finished water at all the locations sampled. While the concentrations of PAH (sum of the 6 PAH) in drinking waters were small, the values found in raw water were as high as 600 ppt. In many cities, all the six representatives of the PAH family were detected. The polynuclear compounds - benzo(a)pyrene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene were among the most frequently occurring PAH (Table 21). Of interest was the finding that fluoranthene, a PAH with relatively higher water solubility (265 ppb at 25°C compared to 10 ppt for BaP, ref. 20,22), was not widely detected. The sum of the six PAH in the finished waters analyzed ranged from 1-15 ppt which are well below the World Health Organization's recommended upper limit of 200 ppt. Water samples derived from Buffalo with

TABLE 20. RESULTS OF ANALYSES OF FIELD SAMPLES

Data presented below is from analysis of a single sample from the selected location

Location and Type of Water	Compound	Total Amt. Detected by TLC (ng)	Total Amt. Detected by GLC (ng)	Conc. in Water by TLC (ppt)
Syracuse				
Finished	FL	N.D.	N.D.	N.D.
	BjF	N.D.	N.D.	N.D.
	BkF	21.5	N.D.	0.4
	BaP	16.3	N.D.	0.3
	IP	N.D.	N.D.	N.D.
	BPR	25.0	N.D.	0.4
	Total of 6 PAH			1.1
	Recovery factor:	0.80		
Buffalo				
Finished	FL	N.D.	N.D.	N.D.
	BjF	N.D.	N.D.	N.D.
	BkF	N.D.	N.D.	N.D.
	BaP	9.6	N.D.	0.2
	IP	N.D.	N.D.	N.D.
	BPR	40.1	N.D.	0.7
	Total of 6 PAH			0.9
	Recovery factor:	0.81		
Raw	FL	N.D.	N.D.	N.D.
	BjF	N.D.	N.D.	N.D.
	BkF	17.1	N.D.	0.6
	BaP	7.6	N.D.	0.3
	IP	N.D.	N.D.	N.D.
	BPR	112.8	N.D.	3.8
	Total of 6 PAH			4.7
	Recovery factor:	0.61		
Pittsburgh				
Finished	FL	N.D.	N.D.	N.D.
	BjF	18.0	N.D.	0.3
	BkF	11.0	N.D.	0.2
	BaP	22.1	N.D.	0.4
	IP	73.3	N.D.	1.2
	BPR	43.6	N.D.	0.7
	Total of 6 PAH			2.8
	Recovery factor:	0.82		
Raw	FL	12250.0	12179.1	408.3
	BjF	1070.0	1507.5	35.7
	BkF	572.5		19.1
	BaP	1262.9	2313.4	42.1
	IP	1812.5	2417.9	60.4
	BPR	1032.5	1492.5	34.4
	Total of 6 PAH			600.0
	Recovery factor:	0.67		

continued

Table 20. (cont'd) Results of Analyses of Field Samples

Location and Type of Water	Compound	Total Amt. Detected by TLC (ng)	Total Amt. Detected by GLC (ng)	Conc. in Water by TLC (ppt)
Huntington				
Finished	FL	144.8	N.D.	2.4
	BjF	20.5	N.D.	0.3
	BkF	11.0	N.D.	0.2
	BaP	27.4	N.D.	0.5
	IP	72.6	N.D.	1.2
	BPR	147.5	N.D.	2.5
	Total of 6 PAH			7.1
	Recovery factor:	0.85		
Raw	FL	704.9	1140.0	23.5
	BjF	150.3	N.D.	5.0
	BkF	109.2	N.D.	3.6
	BaP	169.2	N.D.	5.6
	IP	285.1	N.D.	9.5
	BPR	322.2	N.D.	10.7
	Total of 6 PAH			57.9
	Recovery factor:	0.75		
Endicott				
Finished	FL	259.4	331.5	4.3
	BjF	9.7	N.D.	0.2
	BkF	N.D.	N.D.	N.D.
	BaP	13.7	N.D.	0.2
	IP	42.6	N.D.	0.7
	BPR	171.3	N.D.	2.9
	Total of 6 PAH			8.3
	Recovery factor:	0.89		
Hammondsport				
Finished	FL	N.D.	N.D.	N.D.
	BjF	17.0	N.D.	0.3
	BkF	8.3	N.D.	0.1
	BaP	17.9	N.D.	0.3
	IP	56.7	N.D.	0.9
	BPR	115.8	N.D.	1.9
	Total of 6 PAH			3.5
	Recovery factor:	0.83		

continued

Table 20. (cont'd) Results of Analyses of Field Samples

Location and Type of Water	Compound	Total Amt. Detected by TLC (ng)	Total Amt. Detected by GLC (ng)	Conc. in Water by TLC (ppt)
Philadelphia				
Finished	FL	532.6	541.1	8.9
	BjF	N.D.	N.D.	N.D.
	BkF	N.D.	N.D.	N.D.
	BaP	17.4	*	0.3
	IP	103.6	N.D.	1.7
	BPR	237.4	N.D.	4.0
	Total of 6 PAH			14.9
	Recovery factor: 0.73			
	* the RRT value was slightly different than BaP suggesting a mixture of BaP with some other compound. The amount quantitated using BaP standard was 2692 ng.			
Raw	FL	3430.0	3743.1	114.3
	BjF	1277.5	2138.9	42.6
	BkF	990.0		33.0
	BaP	1232.9	*	41.1
	IP	2172.5	3166.7	72.4
	BPR	1452.5	1986.1	48.4
	Total of 6 PAH			351.8
	Recovery factor: 0.72			
	*The RRT value was slightly different than BaP suggesting a mixture of BaP with some other compound. The amount quantitated using BaP standard was 7279 ng.			
New York City				
Finished	FL	N.D.	N.D.	N.D.
	BjF	70.2	N.D.	1.2
	BkF	39.1	N.D.	0.7
	BaP	32.5	N.D.	0.5
	IP	130.5	N.D.	2.2
	BPR	106.8	N.D.	1.8
	Total of 6 PAH			6.4
	Recovery factor: 0.79			
Lake George				
Finished	FL	N.D.	N.D.	N.D.
	BjF	20.6	N.D.	0.3
	BkF	8.0	N.D.	0.1
	BaP	16.4	N.D.	0.3
	IP	54.5	N.D.	0.9
	BPR	158.4	N.D.	2.6
	Total of 6 PAH			4.2
	Recovery factor: 0.66			
New Orleans ?				
Finished#	FL	N.D.	N.D.	N.D.
	BjF	N.D.	N.D.	N.D.
	BkF	N.D.	N.D.	0.6
	BaP	37.8	N.D.	1.6
	IP	97.0	N.D.	N.D.
	BPR	N.D.	N.D.	2.2
	Total of 6 PAH			
	Recovery factor: 0.66			
	# Water sampled at a motel in old section of New Orleans.			

N.D.: Not Detected

? : Values subjected to error because the sorbent layer on developed plates was accidentally scraped.

TABLE 21. FREQUENCY OF OCCURRENCE OF VARIOUS PAH IN TEN DRINKING WATERS EXAMINED

PAH	Number of drinking waters showing the detectable levels of PAH	% Drinking waters showing the presence of each PAH
FL	3	30
BaP	10	100
BkF	7	70
BjF	6	60
IP	7	70
BPR	9	90

TABLE 22. UNIDENTIFIED LUMINESCENT SPOTS FROM EACH SAMPLE

Type of sample	Total No. of unidentified spots	No. of unidentified spots with substantial luminescence
Syracuse finished water	1	None
Buffalo finished water	1	None
Buffalo raw water	3	1
Pittsburgh finished water	3	1
Pittsburgh raw water	5	1
Huntington finished water	2	1
Huntington raw water	7	4
Endicott finished water	3	2
Hammondsport finished water	2	1
Philadelphia finished water	2	2
Philadelphia raw water	5	2
New York finished water	6	5
Lake George finished water	5	3
New Orleans finished water	4	3

Lake Erie as the source showed surprisingly lower level of PAH. It is difficult to explain the reason for the lower level of PAH at this location than those determined in ground water at Endicott. In this regard it should be mentioned that the Buffalo samples were collected during the severe snowstorm period in that area.

Figure 18 represents the spectra of PAH identified in selected water supplies along with the spectra of the model compounds. In each case, the solid line represents the model compound, whereas the dashed lines represent the TLC spot having the same R_B value and fluorescence color as the model compound. The superimposed spectra for most samples were significantly similar. The absence of the fine structure compared to the model spectra noted in some instances may be due to low extinction coefficient of these line(s) and low concentration of the compound in the spot. This was proven with model compounds with succeedingly lower concentrations. The fluorescence excitation and emission peaks were not obliterated or distorted confirming the presence of a single compound in each spot. The fact that the fine structure did not even flatten out is suggestive of the absence of any unseparated alkylated compounds in the spots. It should be pointed out that in quantitating the results by fluorescence, the error will be comparatively higher when the compounds possess low fluorescence quantum yield as in the case of IP.

In the Philadelphia raw-and-finished water samples, the GLC peak corresponding to benzo(a)pyrene was slightly shifted from the RRT value of the model compound. This is an indication of the presence of a mixture of compounds with very close RRT values and the inability of the GLC column to accomplish the proper separation. Consequently, the amounts of benzo(a)pyrene calculated from GLC values, are erroneous. The separation of this mixture was complete on the TLC plate and the values derived from spectrofluorometric analysis are reliable.

In addition to the 6 PAH identified and quantitated, a number of other unidentified luminescent spots appeared on the TLC plates of various samples. Table 22 lists the total number of unidentified luminescent spots obtained from each sample. Spectrofluorometric identification of these spots could not be made because no matching spectra of known compounds could be located. Examination of the fluorescence emission- and excitation spectra of the unknowns with substantial luminescence revealed that in many instances the same compound was present in many waters. Fluorescence emission- and excitation spectra of selected unknown compounds are given in Figure 19. Selected spots were scrapped off from the plates and subjected to electron impact mass spectroscopic analysis. The results were, however, inconclusive which was attributed to the presence of a large number of background impurities. Either the samples were contaminated during scrapping or impurities were picked up from TLC plate coating during solvent elution. Further efforts to identify these compounds could not be carried out because of the nonavailability of more samples.

The data on the levels of PAH in finished and raw water show that a considerable reduction in the concentration of PAH occurs during water treatment. It is unclear if the decrease is due to actual removal, or transformation of PAH to other products. The treatment process at Hays Mine Treatment Plant at

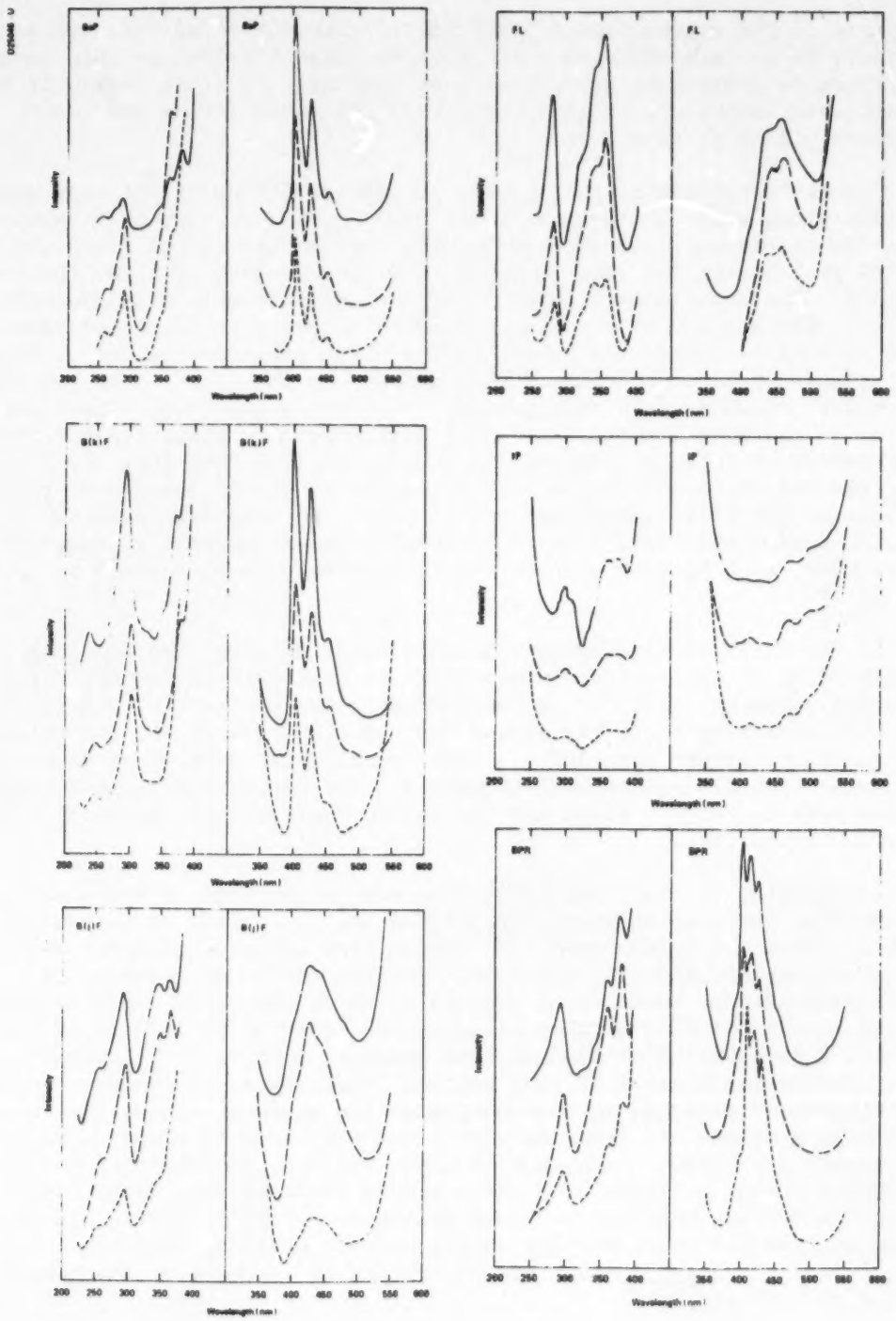

Figure 18. Fluorescence emission and excitation spectra of model PAH and those identified in Huntington, W. Va. water samples.

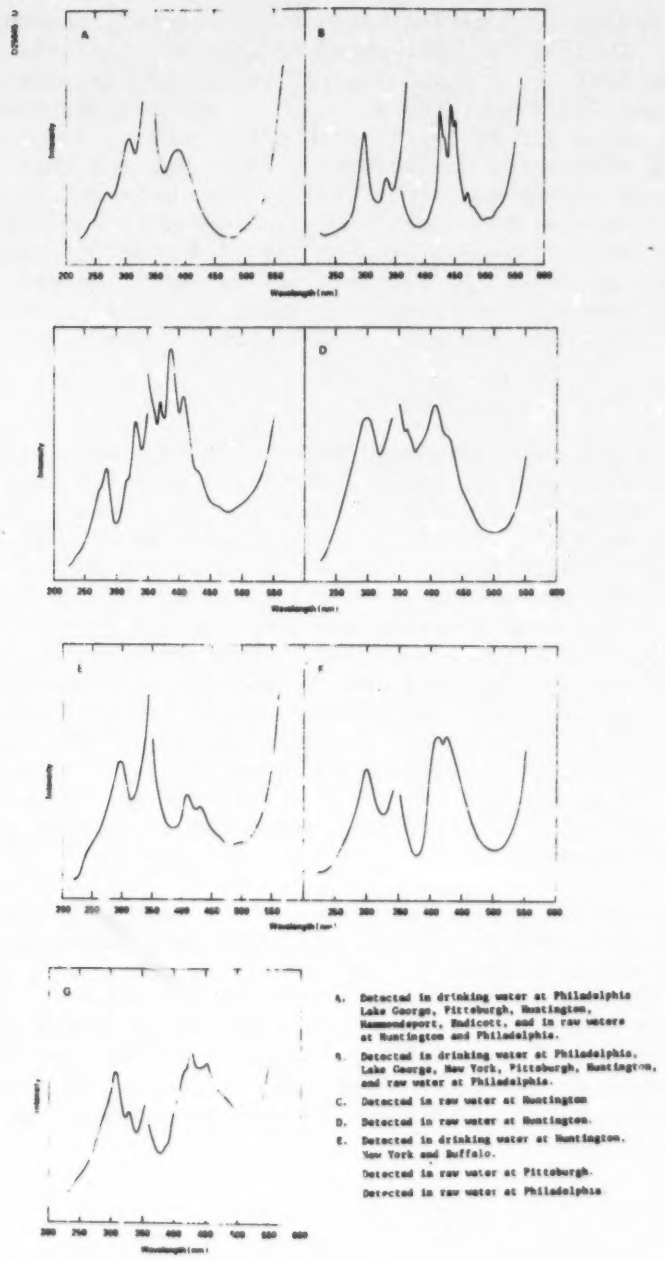
A.
Detected in drinking water at Philadelphia Lake George, Pittsburgh, Huntington, Hammondsport, Endicott, and in raw waters at Huntington and Philadelphia.
B.
Detected in drinking water at Philadelphia, Lake George, New York, Pittsburgh, Huntington, and raw water at Philadelphia.
C.
Detected in raw water at Huntington
D.
Detected in raw water at Huntington.
E.
Detected in drinking water at Huntington, New York and Buffalo.
Detected in raw water at Pittsburgh.
Detected in raw water at Philadelphia

Figure 19. Fluorescence emission and excitation spectra of selected unknown compounds.

Pittsburgh appeared to have the highest PAH removal/transformation efficiency (Table 23 A). This may be attributed to the fact that the water treatment at this plant involved two stages of activated carbon treatment: 1st stage-powdered carbon; 2nd stage-granular carbon, whereas treatment at other plants involved only one stage activated carbon treatment. Comparison of the removal/transformation efficiency of individual PAH suggested that fluoranthene, indeno(1,2,3,-cd)pyrene and benzo(ghi)perylene were not as effectively removed/transformed as benzo(a)pyrene, and benzo(j)- and (k)fluoranthene (Table 23 B). No correlation between the efficiency of removal/transformation and molecular weight of compound was evident. Correlation between removal/transformation efficiency and water solubility could not be made because solubility data for all the PAH were not available.

Conclusions

The sampling method developed for the collection of six PAH from raw and finished waters and the analytical method used for quantitation have been successfully employed for field monitoring. It provides an excellent routine method for the analysis of PAH. The recovery of the six PAH by the method is almost quantitative. The addition of ^{14}C-BaP as internal standard to the foam plugs upon arrival in the laboratory allows determination of the recovery factor for each analysis and thus account for any losses of PAH during elution, clean-up and analysis. Whenever possible, quantitation of each sample should be performed both by GLC-FID and TLC-spectrofluorometric method. This will provide a crosscheck of the values derived. However, in samples containing PAH below the detection limit of FID, only TLC-spectrofluorometric method can be applied for quantitation. This method is capable of detecting PAH at sub-ppt levels from both raw and treated drinking waters. The detection limit of the method can be further improved by increasing the sample volume.

With the application of this method, six representatives of the polynuclear family and several unknown compounds were detected in ppt range at all the water supplies sampled. The levels detected were well below the World Health Organization's recommended limit of 200 ppt. Health hazards to man from the presence of such low levels of PAH in drinking water are not clearly understood. A considerable reduction in the concentration of PAH was noted as a result of water treatment. It is unclear if PAH are actually removed, deactivated or transformed to more carcinogenic product.

TABLE 23. EFFICIENCY OF REMOVAL/TRANSFORMATION OF PAH IN WATER TREATMENT

A. REMOVAL/TRANSFORMATION EFFICIENCY OF VARIOUS TREATMENT PLANTS

Sampling location	Total concn. of the 6 PAH (ppt)		% Removal/ transformation
	Raw water	Finished water	
Pittsburgh, Pa.	600.0	2.8	99.5
Philadelphia, Pa.	351.8	14.9	96.0
Huntington, W.Va.	57.9	7.1	88.0
Buffalo, N.Y.	4.7	0.9	81.0

B. REMOVAL/TRANSFORMATION EFFICIENCY FOR EACH INDIVIDUAL PAH

PAH	% Removal/transformation			
	Pittsburgh	Philadelphia	Huntington	Mean
FL	100	92	90	94.0
BaP	99	99	91	96.3
BkF	99	100	94	97.6
BjF	99	100	94.5	97.8
IP	98	98	87	94.3
BPR	98	92	77	89.0

SECTION IX

LABORATORY EVALUATION OF ACTIVATED CARBON FOR ADDITION/REMOVAL OF PAH

Since the use of granular activated carbon beds for general organic removal in water treatment is fairly widespread, studies were undertaken to determine the ability of activated carbon to remove and/or add PAH. A Barnstead organic removal cartridge (catalog No. 0812) was evaluated at manufacturer's recommended flow rate and life time. Sixty liters of the laboratory tap water was passed through the cartridge and PAH were determined in influent and effluent using the polyurethane plug procedure. The findings showed that no detectable quantities of PAH were leached from the cartridge. The experiment failed to provide information on the ability of the cartridge to remove PAH because the level of PAH in the influent was very low. Further work is necessary for assessment for activated carbon as a method of treatment for removal of PAH.

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APPENDIX

STANDARD OPERATING PROCEDURE

Subject: Monitoring of Polynuclear Aromatic Hydrocarbons (PAH) in Water with Polyurethane Foam Plugs

A. Applicability of the Method

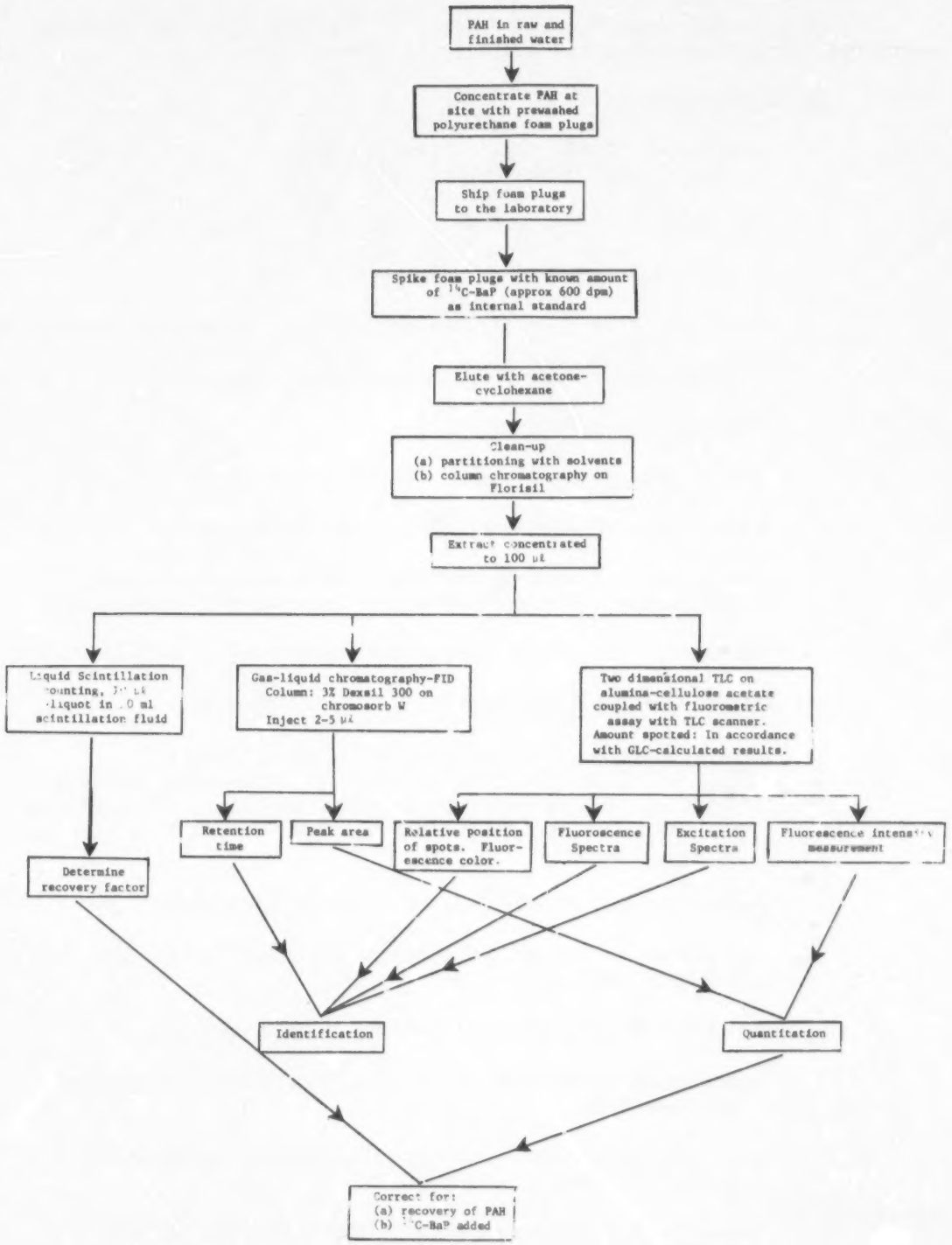
The method described is applicable to drinking waters and their raw water sources. Raw waters containing as much as 102 mg/l of suspended solids and 2.4 g/l dissolved solids have been successfully analyzed. The efficiency of retention has been determined to be >90% in the concentration range of 0.002-25 ppb. The sample volume generally is 60ℓ for drinking water, and 30ℓ for raw water. The method described is for these volumes of water. However depending upon the concentration of PAH suspected, sample volume can be increased or decreased.

Since it is impossible to determine all PAH, analysis has been restricted to the following 6 representatives of the whole group. The World Health Organization recommends analysis of these PAH in drinking waters.

Fluoranthene
Benzo(a)pyrene
Benzo(ghi)perylene
Benzo(k)fluoranthene
Benzo(j)fluoranthene
Indeno(1,2,3-cd)pyrene

B. Flow Chart

C25187-U


PAH in raw and finished water
Concentrate PAH at site with prewashed polyurethane foam plugs
Ship foam plugs to the laboratory
Spike foam plugs with known amount of ^{14}C-BaP (approx 600 dpm) as internal standard
Elute with acetone-cyclohexane
Clean-up
(a) partitioning with solvents
(b) column chromatography on Florisil
Extract concentrated to 100 µl
Liquid Scintillation counting, 5" µl aliquot in 10 ml scintillation fluid
Gas-liquid chromatography-FID
Column: 3% Dexsil 300 on chromosorb W
Inject 2-5 µl
Two dimensional TLC on alumina-cellulose acetate coupled with fluorometric assay with TLC scanner.
Amount spotted: In accordance with GLC-calculated results.
Determine recovery factor
Retention time
Peak area
Relative position of spots. Fluorescence color.
Fluorescence Spectra
Excitation Spectra
Fluorescence intensity measurement
Identification
Quantitation
Correct for:
(a) recovery of PAH
(b) ^{14}C-BaP added

C. Experimental

1. Materials & Reagent: The sources of the materials and reagents needed for PAH concentration and analysis are given below.

PAH Concentration

Foam plugs (Trade name: Identiplugs)
45mm x 45mm: VWR Scientific

Chromaflex columns (25mm) & adapters: Kontes Glass Co.

Oscillating type pumps: Scientific Product Inc.

Haake Model FE thermostated circulator: Scientific Product Inc.

Gilmont flowmeter: Scientific Product, Inc.

Electric timer: local store

Linear propylene Jerrican: Scientific Product Inc.

Right angle thermometer: New Brunswick Scientific Co.

Water trap for introducing right angle thermometer for continuous monitoring of water temperature: custom made

Glass coil (10 ft. x 6 mm): custom made

PAH Analysis by TLC-Fluorometry

PAH Standards: Fluoranthene, Benzo(ghi)perylene, Indeno(1,2,3-cd)pyrene, and Benzo(a)pyrene from Aldrich Chemical Co.; Benzo(j)fluoranthene and Benzo(k)fluoranthene from Dr. J.L. Monkman, Air Pollution Control Directorate, Ottawa, Canada.

Benzo(a)pyrene (7,10-^{14}C): California Bionuclear Corp.

Acetylated cellulose (40%), Aluminum oxide G, type E: Brinkman Instruments Inc.

Florisil (60-100 mesh): Matheson Coleman and Bell Inc.

Spectrophotofluorimeter with Thin Film Scanner: American Instrument Co.

Solvents, Distilled in Glass: Mallinkrodt Chemical Co. and Burdick Jackson Lab Inc.

Chemicals for preparing scintillation fluid: Packard Instrument Co.,

6.5 ml calibrated tubes (each of the last four tenth ml is calibrated to one hundredth ml): Fisher Scientific

PAH Analysis by Gas-liquid Chromatography

6 ft. x 1/8 in. Stainless steel column packed with 3% Dexsil 300 on chromosorb W 100/120 mesh: Altech Associates Inc.

Aluminum foil backed septa (Metasep): Altech Associates Inc.

Other reagents and material: Same as described under TLC-Fluorometric analysis.

2. Sampling System

The sampling system consists of four parts (see Figure)

(a) Thermostated circulator with glass coil emmersed inside the reservoir housing through the opening of the cover plate.

(b) A 18" x 12" x 1/2" plywood piece to which are mounted two column stands, water trap equipped with a thermometer, two oscillating type pumps and a variac to control pumping speed, and flowmeter. The components are connected such that water travels in the order: column → water trap → pumps → flowmeter. The connections up to the foam columns are made with glass tubing as much as possible and only where necessary with tygon tubing. The type of tubing used for connections from this point onward is immaterial.

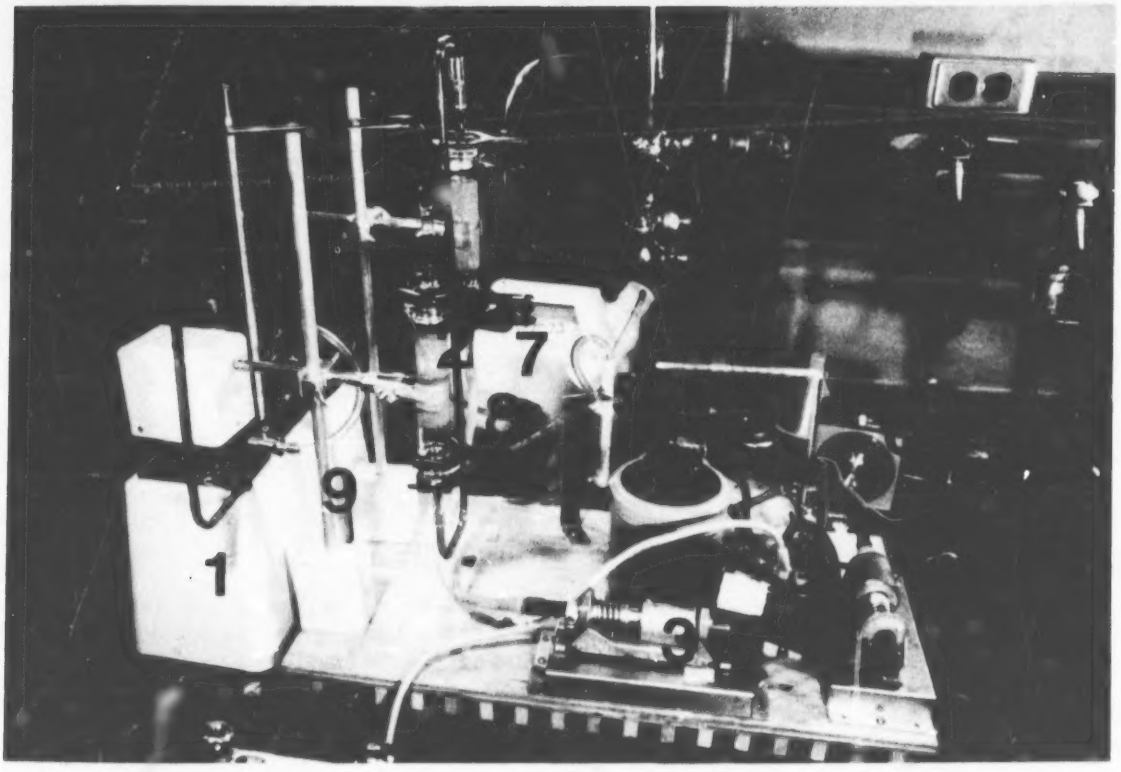
(c) Jerrican, 20 liter capacity.

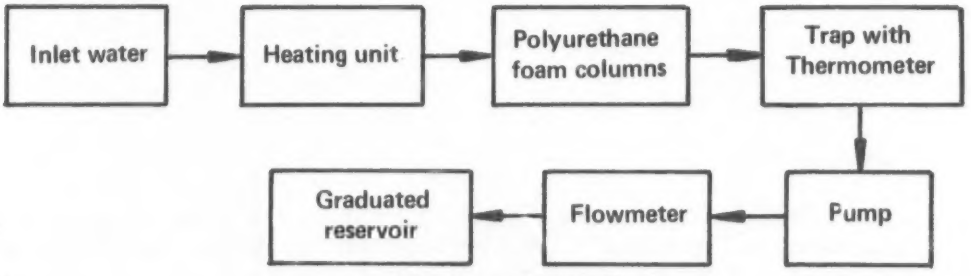
(d) Electric Timer.

3. Procedure

(a) Prewashing of foam plugs: Introduce two foam plugs in each Chromaflex column, and prepare 6 such columns for concentrating PAH from 60ℓ of drinking water or 30ℓ raw water. Wash each column with 30 ml acetone, 125 ml benzene, again with 40 ml acetone and finally with 250 ml distilled water. Wash these foams with an additional 500 ml distilled water at 60°C and squeeze the plugs free of any organic solvents by application of suction. The columns are now ready for passing water for concentration PAH.

(b) Concentration of PAH: The sampling units and washed foam columns are transported to sampling site. Two foam columns are clamped to the column stands in part 'b' of the sampler (see Fig. 1), and are connected in series with the help of glass tubing. The water from the tap which is to be sampled is continuously run, water comes into a beaker from where it is pumped into the sampler. Water is brought to 62 $\pm$ 2°C in the thermostated circulator prior to passing through foam columns.





Apparatus used for concentrating polynuclear aromatic hydrocarbons from water: 1 thermostated circulator, 2 foam columns, 3 oscillating pump, 4 water trap with thermometer, 5 flowmeter, 6 timer, 7 graduated 20ℓ Jerrican, 8 glass beaker, 9 removable column stand.

A water flow rate of 250 ± 20 ml/min is maintained by adjusting the pumping speed with the help of a variac. The thermometer and flowmeter readings are routinely checked throughout the sampling period and proper adjustments in the pumping speed and thermostat are made when necessary.

The effluent from the sampler is collected in a graduated container (20ℓ capacity Jerrican) for determining the volume of water sampled. The direction of flow of water through the complete set-up is depicted in Figure 1. Foam columns are changed every 20ℓ in case of finished water, and every 10ℓ in case of raw water. The unit may be connected to a timer which has been set for appropriate time, for convenience.

(c) Shipment of Foam Columns: PAH are sufficiently stable on foam plugs that no special handling except wrapping columns with aluminum foil is necessary for transportation of foam columns to the laboratory following sampling. However, it is recommended that transit time not exceed 7 days and foam columns be shipped in styrofoam containers cooled with reusable ice packs.

(d) Addition of Internal Standard: Carbon-14 labeled benzo(a) pyrene is employed as internal standard in the method of analysis. A known amount of ^{14}C (approx. 600 dpm) is added to one of the foam columns and then elution of PAH is carried out as described below. All the work from this step on should be done in subdued lighting.

(e) Elution of PAH: Wash each column with 30 ml acetone and 125 ml cyclohexane at a flow rate of 5-10 ml/min into a separatory funnel. Add 50 ml distilled water to the eluate, shake the contents thoroughly and let it stand till the layers are well separated. Disregard the lower aqueous layer and transfer the organic layer into a round bottom flask. Concentrate the layer to about 10 ml with a rotary evaporator maintaining the temperature at 40°C and controlling the vacuum such that no bubbling takes place.

(f) Clean-up: Transfer the extract along with two 10 ml cyclohexane washes into 250 ml separatory funnel. Wash it twice with 60 ml 4:1 methanol-water and twice with 60 ml distilled water. Disregard the bottom methanol water phase in each case. Shake the cyclohexane layer with 20 ml dimethylsulfoxide and let it settle, withdraw the DMSO layer to another 250 ml separatory funnel. Repeat the DMSO extraction two more times and the combined dimethylsulfoxide extract is diluted with water (120 ml). PAH from this phase are extracted with cyclohexane (2 x 40 ml). Wash the combined cyclohexane phase with water (2 x 60 ml) and dehydrate it by passing it through anhydrous sodium sulfate which had been prewashed with cyclohexane. Wash the separatory funnel with cyclohexane (3 x 5 ml) and add the washings to the sodium sulfate bed. Collect the cyclohexane in a 200 ml round bottom flask and concentrate it to 5 ml with a rotary evaporator.

Further purification of the extract is achieved by column chromatography on Florisil. Make a slurry of 8 gms of preactivated 60-100 mesh Florisil in methanol and transfer into a 1.5 x 30 cm column. The bed is further washed with methanol (100 ml) and 100 ml 1:1 n-hexane and benzene. Activate the column by placing it in an oven at 130°C for at least 4 hours. Following activation wash the bed with 100 ml benzene. Transfer the PAH

containing cyclohexane layer on Florisil bed with the help of a Pasteur pipet. Wash the round bottom flask three times with 5 ml benzene and add the washings to the Florisil column. Elute PAH with 125 ml benzene at a flow rate of 5 ml/min. Concentrate the eluate to about 2-3 ml by rotary evaporation and transfer the contents with adequate washing to a calibrated tube. Concentrate the layer further to 0.1 ml with prepurified grade nitrogen. The extract is now ready for analysis by gas-liquid or thin layer chromatography.

(g) Determination of Recovery Factor: A 10 µl aliquot of the concentrate is assayed for radioactivity in a liquid scintillation system. Scintillation fluid is prepared by mixing 5g of 2,5-diphenyloxazole (PPO) and 0.15 g 2,2'-phenylene bis-(5-phenyl)oxazole (POPOP) in a liter of scintillation grade toluene. Knowing the ^{14}C-activity originally added to the foam plugs allows calculation of the recovery factor.

(h) Analysis: It is recommended that analysis be carried out using both the gas liquid chromatography with FID detection and thin layer chromatography with spectrophoto-fluorometric detection. The limitation of the gas chromatographic analysis of PAH are: (i) With the column used in the present study and most other columns, it fails to separate isomeric PAH compounds (for example, benzo(k)fluoranthene and benzo(j)fluoranthene give one peak), (ii) The limit on the sample volume that can be injected makes detection of low levels of PAH impossible. These problems are overcome when the analysis is carried out by thin layer chromatography coupled with fluorometry. On the other hand, TLC-fluorometric analysis is more time consuming and probably not as quantitative as gas liquid chromatography.

(i) Analysis by Gas-Liquid Chromatography: Analysis is performed using a gas chromatogram equipped with a dual flame ionization detector system and linear temperature programmer. The experimental conditions used in gas chromatographic analysis of PAH are as follows:

Column: 6 ft x 1/8" stainless steel packed with 3% Dexsil 300 on Chromosorb W, 100-120 mesh (two columns operated in differential mode).
Carrier gas (nitrogen) flow rate: 30 ml/min.
Detector temperature: 300°C
Injection port temperature: 250°C
Column oven temperature programmed as follows:
- Initial temperature: 200°C
- Initial delay: 2 min.
- Program: 4°C/min.
- Final temperature: 290°C
- Final delay: 8 min.

Standard mixture: 100 ppm of each PAH in benzene (inject 1-2 µls).

The septa used must be aluminum foil backed (Metasep) and preconditioned for 12 hours at 250°C.

(ii) Analysis by Thin Layer Chromatography coupled with Fluorometry:

Preparation of Plates: Mix 28g aluminum oxide G type E, 12g 40% acetylated cellulose and 2g $CaSO_4 . 2H_2O$, 200 mesh (activated for 2 hours at

130°C) with 83cc of 95% ethanol on a magnetic stirrer for 5 min. The resultant slurry is spread to a thickness of 250 μm on eight 20 x 20 cm glass plates using a coating apparatus. The plates are air dried for 1/2 hr and activated for 30 min. at 80°C. Plates are stored in a desicator.

Sample Application: Appropriate aliquot of the concentrate is applied with the help of a syringe in one corner of the TLC plate about 2 cm from the two sides. The spot is dried with prepurified grade nitrogen. The concentration of PAH as revealed by GLC analysis may serve as a guide to determine the amount that needs to be spotted. Best resolution is obtained in the concentration range of 20-100 ng compound/spot.

Development of Plates: Plates are developed in the first direction with n-hexane:benzene (4:1) (developing time approx. 30 min). After drying the plate is rotated by 90°C and developed in the second direction with methanol:ether:water (4:4:1) (developing time approx. 2 hr).

Location of Spots: The dried chromatogram is taken into a dark room and fluorescent spots are visualized with low intensity U.V. illuminators. The boundaries of spots are marked with a clean, sharp needle.

Identification of Spots: Tentative identification of the spot is based on (a) fluorescence color and (b) relative position on the plate and comparison with reference chromatogram.

The fluorescence colors of the 6 PAH are given below:

Benzo(ghi)perylene	violet
Benzo(j)fluoranthene	yellow-orange
Benzo(k)fluoranthene	dark blue
Benzo(a)pyrene	violet
Fluoranthene	light blue
Indeno(1,2,3-cd)pyrene	yellow-green

The identity of each spot is confirmed by obtaining fluorescence emission- and excitation spectra. The spectra are run directly on the plate with the help of spectrofluorometer equipped with a thin film scanner and a X-Y recorder. The position of the scanner is adjusted to obtain the maximum fluorescence signal at a desired spot. Excitation and emission spectra are then obtained using the wavelengths given below:

Compound	Emission spectra λ for excitation (nm)	Excitation spectra λ for emission (nm)
Benzo(ghi)perylene	300	430
Benzo(j)fluoranthene	300	427
Benzo(k)fluoranthene	300	428
Benzo(a)pyrene	300	427
Fluoranthene	280	458
Indeno(1,2,3-cd)pyrene	300	467

The fluorescence emission and excitation peaks of the WHO recommended polynuclear aromatic hydrocarbons on TLC plates are given below:

Compound	Fluorescence excitation spectra, wavelength, nm	Fluorescence emission spectra, wavelength, nm
Benzo(a)pyrene	263, 293, 363, 382	403, 427, 453
Benzo(ghi)perylene	295, 362, 380	404, 416, 426
Benzo(j)fluoranthene	293, 347, 365	427, 450
Benzo(k)fluoranthene	240, 300, 376	403, 428, 457
Fluoranthene	278, 342, 354	435, 458
Indeno(1,2,3-cd)pyrene	297, 362, 375	467, 497

Quantitation: Quantitative analysis of the separated compounds is performed by scanning each spot for fluorescence intensity with the help of a strip chart recorder. The area of the recorded peak is proportional to the amount of substance present. Measurement of peak areas is made with a planimeter. The concentration of PAH in unknown sample is determined from the calibration curve.

The experimental conditions used in fluorescence intensity measurement are as follows:

Recorder (50 mv) chart speed: 4"/min.
Scanner cycle time: 2 min.
Excitation wavelength: 365 nm
Fluorescence wavelengths (nm)

Benzo(ghi)perylene	416
Benzo(j)fluoranthene	427
Benzo(k)fluoranthene	428
Benzo(a)pyrene	427
Fluoranthene	458
Indeno(1,2,3-cd)pyrene	467

Preparation of Reference Chromatogram and Calibration Curve:

Prepare a 100 ppm stock solution of each of the six PAH. Mix 5 ml of the stock solution of fluoranthene and 1 ml each of the other stock solutions in a small glass stoppered flask. The test solution now contains 50 ng of fluoranthene, and 10 ng each of the other PAH in 1 µl.

Prepare three reference chromatograms with 2, 5 and 10 µl of the test solution. Scan spots for fluorescence intensity. For each PAH, a calibration curve is set up by plotting emitted fluorescence (area of the peak) at the three concentrations as a function of the amount of the compound applied. Employing one of the above chromatograms obtain reference emission and excitation spectra for each of the PAH.

TLC Foam Blank: During elution of PAH from foam plugs a number of impurities belonging to the foam are also eluted. The clean-up procedure eliminates these impurities to the extent that they are not seen in the gas

chromatographic analysis of the extract. However, since the volume of the extract subjected to thin layer chromatography is much larger, traces of fluorescent impurities (0-3 spots, depending upon the volume of the concentrate spotted) show up in the thin layer chromatography. This necessitates running a TLC-foam blank and with its help marking the spots of foam origin in the test chromatogram and correcting for background fluorescence. To prepare TLC-foam blank, 12 prewashed foam plugs are extracted, the extract cleaned-up and subjected to thin layer chromatography as described above.

(iii) Correction of the Values Determined by Analysis: In order to calculate the actual amount of PAH present in water samples, the amount determined by GLC or TLC analysis should be corrected for the recovery factor as determined earlier. In addition, the benzo(a)pyrene values should be further corrected for the amount of ^{14}C-benzene(a)pyrene added as internal standard.